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Acute and Chronic Toxicity of Cadmium to the Fathead Minnow (*Pimephales promelas*)

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PICKERING, Q. H., AND M. H. GAST. 1972. Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*). J. Fish. Res. Bd. Canada 29: 1099–1106.

Two continuous-flow bioassays on the chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas* Rafinesque) indicated that the maximum acceptable toxicant concentration (MATC) for this species is between 57 and 37 μg Cd/liter. The experimental concentration of 57 μg Cd/liter decreased survival of the developing embryos, the embryos being the most sensitive life stage. At lower concentrations of 37 to 4.5 μg Cd/liter, no adverse effect on survival, growth, or reproduction was found. Application factors based on the acute bioassays are discussed in relation to the great variation in toxicity values.

PICKERING, Q. H., AND M. H. GAST. 1972. Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*). J. Fish. Res. Bd. Canada 29: 1099–1106.

Nous avons effectué deux épreuves biologiques à flot continu en vue de déterminer la toxicité chronique du Cd à l'endroit du méné à grosse tête, *Pimephales promelas* Rafinesque. Le maximum de concentration toxique acceptable (MATC) du Cd pour le méné à grosse tête se situe entre 57 et 37 μg Cd/litre. La concentration expérimentale de 57 μg Cd/litre diminue la survie des embryons en voie de développement, le stade embryonnaire étant le plus vulnérable. A des concentrations plus faibles de 37 à 4.5 μg Cd/litre, nous n'avons pu détecter d'effets adverses sur la survie, la croissance et la reproduction. La reproductibilité des deux épreuves de toxicité chronique a été excellente; cependant, celle d'épreuves de toxicité aiguë a été pauvre. Nous examinons les conditions d'application basées sur la variation des épreuves de toxicité aiguë.

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IN this study two long-term, continuous-flow bioassays were made to determine the chronic toxicity of cadmium to the fathead minnow, *Pimephales promelas* Rafinesque. The second test was conducted 2 years after the first to allow an evaluation of the precision and repeatability of chronic bioassays with fish and to more precisely bracket the maximum acceptable toxicant concentration (MATC) for cadmium. The MATC is the maximum toxicant concentration that has no adverse effect on sur-

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vival, growth, or reproduction. In addition, acute toxicity studies were conducted to estimate an application factor for cadmium toxicity. Mount and Stephan (1969) proposed that an application factor be calculated by dividing the MATC by the 96-hr TL50, and suggested that this application factor might be applicable to other waters and other species.

Cadmium is found only in minute traces in natural water (Kopp and Kroner 1969). The occurrence of this metal in zinc ores reflects its close chemical relation to zinc (Lingane 1966). It may be found also in wastes from electroplating, ceramic, chemical, and pigmentation plants. Although cadmium is not as common as some of the other heavy metals, it has a high pollution potential because of its high toxicity and long biological half-life. Cadmium concentrations in excess of 10 µg/liter in drinking water is grounds for rejection (Anon. 1962).

Nilsson (1970), Flick et al. (1971), and Friberg et al. (1971) have reviewed the literature on the occurrence of cadmium and its biochemical and toxicological effects. In their review of toxicity of cadmium to fish, McKee and Wolf (1963) indicate that the lethal concentration for fish varies from about 10 to 10,000 µg Cd/liter. On the basis of a continuous-flow test in hard water, Ball (1967) reported a 7-day TL50 for rainbow trout of between 8 and 10 µg Cd/liter.

Methods

In general the methodology for the bioassays was similar to that of Mount and Stephan (1967) and Pickering and Thatcher (1970). The stock solution of cadmium used for the exposures was made by dissolving reagent-grade cadmium sulfate in demineralized water. Diluent water was maintained at an EDTA hardness of 200 mg/liter (as $CaCO_3$) by diluting pond water originating from a spring with carbon-filtered, demineralized Cincinnati tap water. The water in each chamber was maintained at 30 liters, and the flow rate from the diluter gave an average retention time of 4–6 hr. Routine analyses of the test water from these exposure chambers (Table 1) were made according to the methods of the American Public Health Association et al. (1965).

Six pieces of half-tile were placed in each exposure chamber for territorial and spawning sites. During the spawning season these tiles were examined daily for eggs, and after spawning was completed the eggs were removed and counted. Eggs were removed on the day of deposition, and 100 from a single spawning were placed in an incubator (Mount 1968). Hatchability was calculated as the percentage of fry surviving from these eggs incubated for 7 days.

Chronic Test 1

A serial diluter (Mount and Warner 1965) delivered a control and 5 different concentrations of cadmium (Table 2) with a dilution factor of 0.33. The experimental fish were obtained in early November 1965 from ponds at the Newtown Fish Farm, Ohio Division of Wildlife. During the last week of November, 30 fish that averaged 0.23 g were placed randomly into each randomly arranged exposure concentration. During January the number of fish per chamber was reduced to 20 except in the two high concentrations, in which mortality occured. During February the number of fish per aquarium was reduced further to 12 in the control and three low cadmium concentrations. After the fish reached sexual maturity a few excess males were removed to reduce territorial conflict. The exposure was terminated in October 1966.

Illumination was provided by 6 cool-white, 20-w fluorescent lights that gave a constant 16-hr photoperiod. Temperature was recorded continuously and varied between 16 C and 27 C. The mean weekly temperature

Table 1. Chemical and physical characteristics (*mg/liter*) of test water.

Characteristic	Chronic test 1			Chronic test 2		
	No. analyses	Mean	Range	No. analyses	Mean	Range
Dissolved oxygen	183	6.5±1.5[a]	2.2–9.7	372	6.6±1.2[a]	3.5–10.1
*p*H	95	7.7±.20	7.4–8.1	124	7.6±.14	7.2–7.9
Hardness (as $CaCO_3$)	44	201±6.1	187–218	287[b]	204±8.4	186–233
Alkalinity (as $CaCO_3$)	48	161±4.7	153–170	122	154±11	136–174
Acidity (as $CaCO_3$)	48	8±3.7	2–14	122	12±4.3	4–32

[a]One standard deviation of mean.
[b]Daily analysis of diluent water.

TABLE 2. The five concentrations (*μg/liter*) of cadmium used in three tests (values are for weekly determinations on composite samples).

Chronic test 1		Chronic test 2		60-day growth test	
Mean	Range	Mean	Range	Mean	Range
350±23	280–400	110±10	93–140	110±11	96–140
110±8.9	100–130	57±5.2	46–79	54±9.3	30–72
37±3.7	32–48	27±3.4	21–41	29±4.6	23–36
13±1.2	10–16	14±2.1	10–24	15±2.7	10–21
4.5±0.67	3.1–6.8	7.8±1.7	6–15	6.8±1.3	4.8–9
<1		1.0±1.0	0.5–5	1.2±0.95	0.6–2.4

slowly increased from 18 C in the winter to 23 C in April, then varied between 23 C and 26 C for the duration of the test. Difficulty was encountered in maintaining dissolved-oxygen concentrations above 5 mg/liter during April, and it became necessary to aerate. This aeration was continued for the duration of the test.

Daily samples for cadmium analysis were removed from each chamber and 1 liter composited for 7 days. The composite samples were acidifed with five drops of concentrated sulfuric acid. For the three low concentrations and control, the composites were passed through an ion-exchange column (Mount 1968). The absorbed cations, including cadmium, were eluted with 100 mg of 25% redistilled hydrochloric acid and evaporated to dryness. One-hundred-milliliter aliquots of the acidified composites from the two high concentrations were acidified with 5 ml of 50% redistilled hydrochloric acid and evaporated to dryness. The dried samples were dissolved in 5 ml of electrolyte, and the cadmium measured on a Sargent Model XV Polarograph.[1] All samples were calculated on the basis of spiked control water samples treated as described above.

CHRONIC TEST 2

Since the chronic toxicity of cadmium was known at this time, a proportional diluter (Mount and Brungs 1967) was used to deliver a control and five concentrations of cadmium (Table 2) with a dilution factor of 0.5 that more closely bracketed the MATC. This exposure was started with 3-week-old fry that had been reared from eggs produced by fish in the laboratory. A sample of these fry averaged 12 mm in total length and varied from 6 to 14 mm. Eighty fry were placed randomly in each randomly arranged exposure concentration, and the exposure started on October 3, 1967. Thirty days later the fry were counted and measured to determine survival and growth. At this time each exposure chamber was divided into two spawning areas by means of a plastic screen across the middle of the aquarium. The water from the diluter was divided to deliver to each side of the screen. Fifteen fry were returned to each side of the tank except for the high concentration (110 μg Cd/liter), in which only 14 fish had survived. Later in the test excess males were removed to reduce territorial conflict.

One 60-w incandescent bulb was suspended 25 cm above the middle of each chamber. These lights and the fluorescent ceiling lights were controlled by a time switch to give a photoperiod of 10 hr of light initially, 16 hr by the sixth month at monthly increments of 1 hr, and 16 hr for the rest of the test. Mean weekly temperatures decreased from 24 C in October 1967 to 19 C in January, and then increased to 25 C in June, where it remained for the duration of the test.

A growth study of the progeny was conducted in a separate exposure system. Fifty fry from one spawning were placed in exposure chambers at cadmium concentrations (Table 2) similar to those in which the eggs were produced. The mean temperature for the 60-day exposure was 23 C.

For cadmium analysis, 100-ml grab samples were dipped from each test chamber and composited for 5 weekdays. After acidification with one drop of concentrated sulfuric acid per 100 ml, a 200-ml aliquot of each composite was poured into a separatory funnel and extracted three times with 25 ml of 0.1% diethylammonium diethyldithiocarbamate in chloroform (Stary 1964). The samples were shaken for 2 min for each extraction, and the chloroform extract, along with any interfacial cuff from one sample, was collected in a 150-ml beaker. The chloroform extract was evaporated to dryness on a steam bath. After addition of 10 ml of concentrated nitric acid, the samples were evaporated to dryness on a hot plate. The residue was dissolved in 10 ml of 10% acetic acid. The samples were run on a Perkin Elmer[2] Model 303 atomic absorption spectrophotometer against standards in 10% acetic acid. The average of 88 recoveries from spiked samples was 100.2% with a standard deviation of 2.7%.

ACUTE TESTS

Static and continuous-flow tests were used to determine the acute toxicity of cadmium. Two 96-hr

[1]Mention of product does not constitute endorsement by the Environmental Protection Agency.

[2]Mention of product does not constitute endorsement by the Environmental Protection Agency.

static bioassays were conducted according to the methods of the American Public Health Association et al. (1965). Two groups of five immature fathead minnows, averaging 2 g, were each placed in 10 liters of solution. Tests were conducted at 25 C with the same type of water used in the chronic toxicity tests.

Five continuous-flow bioassays were conducted in the same type of water as that used in the chronic tests. The first two tests were conducted with a serial diluter with 10 fish per concentration and were made at the time of the static tests. The last three tests were made with a proportional diluter with 10 fish in each duplicate exposure chamber, after the completion of the second chronic study. These chambers were 30 × 30 × 30 cm and contained 15 liters of water. The flow rate was such that the tank volume per 24 hr was similar to that in the chronic tests. All TL50 values were calculated by graphical interpolation (American Public Health Association et al. 1965).

For the measured concentrations of cadmium, 25-ml samples were collected about 50 mm below the surface with a tipless pipette. These daily samples were composited for the duration of the test, acidified, and run by direct aspiration on an atomic absorption spectrophotometer against standards in distilled water. In addition, some daily grab samples were run as above, and some were filtered through a 0.45 membrane filter to estimate dissolved cadmium. The filter was washed with 10 ml of distilled water and a 50-ml aliquot of the sample before a 50-ml aliquot was filtered, acidified, and run as above.

Statistics

Analysis of variance with an F test of significance ($P = 0.05$) was used for statistical analysis of the data. The Student-Newman-Keuls procedure (Sokal and Rohlf 1969) for comparison among means was used with unequal sample sizes, and Duncan's multiple range test (Hicks 1964) was used with equal sample sizes.

Results

A slow cumulative mortality occurred in the two highest cadmium concentrations in chronic test 1. In the highest concentration (350 µg Cd/liter) only two fish survived the first 5 weeks of exposure, and these two were dead after another 4 weeks. In the second highest concentration (110 µg Cd/liter), the first fish died on the seventeenth day, and ten were dead after 5 weeks. Mortality occurred until the end of the test, when only three females were alive. No fish died in the three lowest test concentrations and control.

The TL50 values (µg Cd/liter) were: 150 for 5 weeks, 89 for 9 weeks, and 68 for 9 months.

In chronic test 2, which began with younger fish, only 18% of the fry survived the first 30 days of exposure to the highest concentration, 110 µg Cd/liter (Table 3). Survival at lower concentrations was similar to that of the control fish. Survival of the progeny of these fish (Table 4) was similar to that of the first generation fish reared in similar concentrations.

TABLE 3. Survival and growth of fathead minnows after 30 days' exposure to various cadmium concentrations (80 fish per concentration).

Measured concentration (µg/liter)	Survival (%)	Length[a] (mm) Mean	Range
110	18	20.1±5.6[b]	11–32
57	63	22.6±5.1	13–34
27	63	21.7±5.8	12–37
14	59	23.7±6.1	14–38
7.8	69	23.0±5.8	12–35
1.0 (control)	70	22.8±6.1	10–38

[a]Mean initial length, 12 mm (range, 6–14).
[b]One standard deviation of mean.

TABLE 4. Survival and growth during 30 and 60 days of fathead minnow fry spawned and reared at various cadmium concentrations (50 fry per concentration).

Measured concentration (µg/liter)	30 days Survival (%)	30 days Length (mm) Mean	30 days Length (mm) Range	60 days Survival (%)	60 days Length (mm) Mean	60 days Length (mm) Range
110	16	17.1±1.8[a]	14–19	16	31.5±2.2[a]	28–34
57	74	19.6±2.4	12–24	68	33.0±4.0	27–40
29	50	20.7±1.6	18–24	50	35.4±2.3	31–40
15	96	19.1±3.1	9–25	96	30.7±3.1	25–36
6.8	66	20.6±2.4	15–25	66	32.4±4.5	22–40
1.2 (control)	66	19.6±3.2	10–23	64	33.0±4.1	19–38

[a]One standard deviation of mean.

The percentage hatchabilities of eggs for 11–23 replicates (100 eggs per test) in chronic test 2 were:

Cadmium concentration (μg/liter)	57	27	14	7.8	1.0 (control)
Hatchability (%)	78.4	93.6	95.4	96.5	94.9

Statistical analysis of the data indicated a significant effect ($P = 0.05$) only at 57 μg Cd/liter. Some eggs were transferred within the exposure concentrations to gain additional information on the effect of cadmium on hatchability. Two spawnings each from the control and 57 μg Cd/liter exposure chambers were transferred to other concentrations. The hatchability of these eggs was similar to that of eggs spawned and incubated in similar cadmium concentrations.

In chronic test 1 there was no spawning at the two high concentrations of 350 and 110 μg Cd/liter. The hatchability of eggs produced and incubated at the lower concentrations (37–4.5 μg Cd/liter) varied between 92 and 100% and was not related to cadmium concentration.

Growth at sublethal concentrations of chronic test 1 was not related to cadmium concentration. Analysis of variance of the individual lengths after 30 days of exposure in chronic test 2 (Table 3) indicated that cadmium had no significant effect ($P = 0.05$) on growth. The growth of fry from cadmium-exposed fish (Table 4) was not related to cadmium concentration.

Spawning in chronic test 1 (Table 5) started on June 18 and continued until the test was terminated on October 19. At sublethal concentrations, egg production was not related to cadmium concentration. In the second test, spawning (Table 5) started on July 3 and was continuous until the bioassay was terminated on December 5. The mean number of eggs per female at 14 μg Cd/liter was significantly different ($P = 0.05$) from that at each of the other test concentrations and control, and that at 110 μg Cd/liter was significantly different ($P = 0.05$) from those at all lower concentrations except 57 μg Cd/liter.

The 96-hr TL50 values of the two static bioassays were 32,000 and 30,000 μg Cd/liter. Toxicity was greater (TL50 values less) in the continuous-flow bioassays (Table 6).

Discussion

The results clearly show that the MATC for cadmium for fathead minnows is between 57 and 37 μg Cd/liter. The data indicate that 57 μg Cd/liter decreased survival of the developing embryos. Hatchability of control eggs transferred to this concentration was similar to that of eggs from fish exposed to this concentration. Most of the mortality occurred during the last 3 days

TABLE 5. Spawning and egg production data for the fathead minnow during chronic exposure tests with various concentrations of cadmium.

Measured concentration (μg/liter)	No. males[a]	No. females[a]	No. spawnings	No. eggs/spawning	No. eggs/female
		Test 1			
110	0	3	0	0	0
37	4	6	34	121	687
13	5	4	31	172	1,331
4.5	5	4	38	112	1,061
<1 (control)	4	5	17	164	559
		Test 2[b]			
110	2,4	2,1	2,8	133,84	133,673
57	4,6	4,7	23,69	119,126	686,1238
27	3,6	6,5	60,40	157,166	1566,1331
14	4,3	3,2	80,55	184,156	4916,4297
7.8	3,3	6,6	77,52	145,178	1864,1545
1.0 (control)	4,4	6,8	48,75	206,138	1645,1291

[a]At end of test.
[b]Values are for two replicates.

TABLE 6. Acute toxicity of cadmium (as TL50 values in micrograms cadmium per liter) for the fathead minnow on three bases.

Test no.	Cadmium concn	Days 1	2	4	6	8
1	Nominal	20000	19000	19000		
	Measured	12000	11000	11000		
2	Nominal	23000	19000	19000		
	Measured	15000	12000	12000		
3	Nominal	14000	10000	10000	10000	
	Measured	8700	6400	6400	6400	
	Dissolved	5400	4600	4600	4600	
4	Nominal	12000	3200	3200	3000	
	Measured	5600	2000	2000	1900	
	Dissolved	2700	1400	1400	1300	
5	Nominal	22000	12000	6000	6000	750
	Measured	12000	7800	4500	4500	450
	Dissolved	6900	4500	2800	2800	340

of incubation. Survival averaged 78% and varied from 35% to hatchability similar to that of the control. However, many of the survivors were inactive and deformed. Apparently, cadmium had an effect on the circulatory system of the embryo; often the heart would be beating, but the RBC would not be circulating. There were many blood clots throughout the vascular system. At cadmium concentrations of 37–4.5 μg/liter no adverse effect on survival, growth, or reproduction was found.

An unexpected effect on reproduction in the second chronic test was the significantly greater (P – 0.05) number of eggs per female at 14 μg Cd/liter than at other concentrations. Also, in the first chronic test the mean number of eggs per female in 13 μg Cd/liter was about twice that of the control. Perhaps this apparent stimulation of egg production at this low cadmium concentration is an example of "sufficient challenge," which Smyth (1967) defines as an apparent benefit from small doses.

The second chronic bioassay indicated high reproducibility of chronic tests and bracketed the MATC more accurately. In both bioassays, a concentration of 110 μg Cd/liter was lethal to the experimental fish. At similar concentrations of 37 μg Cd/liter in the first study and 27 μg Cd/liter in the second, no toxic effects were detected. The intermediate concentration of 57 μg Cd/liter of the second test decreased survival of developing embryos.

No matter how refined a chronic bioassay is conducted and how sophisticated the measurement of effect, the results are only indicative of that species and water quality. For example, the highest tested "no-effect" concentrations of 37 μg Cd/liter (chronic test 1) and 27 μg Cd/liter (chronic test 2) for the complete life cycle of the fathead minnow are greater than the 7-day TL50 of 8–10 μg Cd/liter for the rainbow trout as reported by Ball (1970). Mount and Stephan (1967) have proposed "application factors" for estimating "safe" concentrations for other species and water quality. This application factor is derived from the relations between chronic and acute studies. Mount and Stephan (1969) have suggested further refinements of tests designed to check the validity of the application factor.

The TL50 values for the five acute toxicity bioassays (Table 6) varied greatly for the calculation of an application factor for cadmium. This variation was much greater than would be expected from variability of the fish. Probably some of the variation is due to the chemical reactions of cadmium in the diluent water. In the acute bioassays there was much precipitated cadmium on the bottom and sides of the exposure chambers, in suspension, and floating on the surface. Two sources of variation in the bioassays were the initial high concentration of cadmium and the *p*H of the diluent water. The two tests with the highest initial concentration had the highest TL50 value, and the test in the

lowest initial concentration had the lowest TL50 value. Although the bioassays were conducted with a diluent water of 200 mg/liter hardness as $CaCO_3$, the *p*H values were different. It appeared that cadmium might be more toxic at a higher *p*H. Because of a change of methods in measuring *p*H, it is not possible to evaluate the effect of *p*H more completely. However, R. W. Andrews (personal communication) found that cadmium was more acutely toxic at a higher *p*H.

Another major factor in the variation of TL50 values was the fact that in one of the duplicates mortality varied little over a fourfold range of concentrations. Ball (1967) reported a similar situation for the rainbow trout; the curve relating concentration from 10 to 1000 μg Cd/liter to time of survival could be described by a horizontal line passing through the 95% confidence limits of the median period of survival.

Mount and Stephan (1969) proposed that for the development of application factors the 96-hr TL50 should be calculated on the basis of measured toxicant concentration, but suggested that means must be devised to determine and measure what portion of the toxicant is actually toxic. Dissolved cadmium was measured in three of the acute bioassays as an approach to explain the variation in acute toxicity. The TL50 values were calculated on the basis of nominal, measured total, and measured dissolved concentrations. The variations in TL50 values were about the same for these three measurements (Table 6).

Sprague (1969) suggested that, whenever possible, acute bioassays should be continued until the toxicity curve becomes essentially parallel to the time axis, indicating a lethal threshold concentration. Eaton (1970) proposed that the definition of the application factor be changed from MATC/96-hr TL50 to MATC/lethal threshold concentration. In many cases this change will not affect the numerical value of the application factor, but overall it might allow a more valid comparison of application factors between species, toxicants, and chemical conditions.

All of the acute bioassays reported herein appeared to reach a lethal threshold concentration in 2–6 days. One of the bioassays, which continued for 8 days, had increased mortality after 6 days, indicating the possibility of two distinct toxic syndromes.

Because of the cumulative mortality in the acute tests, it is not possible to propose a firm application factor for cadmium. When the mean of the five 96-hr TL50 values (measured concentrations) is used, the application factor for cadmium is between 37/7200 and 57/7200, or 0.005 and 0.008. If the 8-day TL50 value (measured concentrations) is used as an approximation for lethal threshold concentration, the application factor is between 37/450 and 57/450, or 0.08 and 0.13.

The rainbow trout is a very sensitive species to cadmium (Ball 1967). Other salmonids also appear to be very sensitive to cadmium (D. A. Benoit and G. R. Bouck personal communication). Studies are being made at the National Water Quality Laboratory, Duluth, Minn., to determine the application factor for cadmium using the brook trout as the test animal. A long-term study has been completed at the Newtown Fish Toxicology Laboratory, Cincinnati, Ohio, in which the chronic toxicity and the application factor for cadmium have been determined using the bluegill. When these studies are completed, it should be possible to have a better understanding of the validity of the application factor for cadmium.

A supplemental approach to estimating an upper limit for the "no-effect" concentration would be to obtain information on acute lethality of cadmium to important species using the most sensitive life stage. The developing embryo of the fathead minnow was the most sensitive stage in the chronic exposure to cadmium. Exposure of the spawning adults apparently had little, if any, effect on the toxicity of cadmium to the developing embryos. Thus, the acute toxicity could be used as an early indicator of response for the chronic exposure.

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Effects of Temperature on Growth and Survival of Young Brook Trout, *Salvelinus fontinalis*

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McCormick, J. H., K. E. F. Hokanson, and B. R. Jones. 1972. Effects of temperature on growth and survival of young brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Canada 29: 1107–1112.

Instantaneous rates of growth, mortality, and net biomass gain were determined for alevin through juvenile brook trout reared for 8 weeks at six constant temperatures: 7.1, 9.8, 12.4, 15.4, 17.9, and 19.5 C. Growth rates were maximum between 12.4 and 15.4 C. Mortality rates increased between 15.4 and 17.9 C and were maximum between 17.9 and 19.5 C. The net rates of biomass gain were maximum between 12.4 and 15.4 C.

Median upper thermal tolerance limits (TL50 values) were determined for newly hatched and swim-up alevins. Tolerance did not increase in newly hatched alevins with acclimation to temperatures from 2.5 to 12 C. The upper 7-day TL50 for newly hatched alevins acclimated over this range of temperatures was 20.1 C. The swim-up alevins showed both an increase in temperature tolerance with acclimation temperatures between 7.5 and 12 C and an increase in tolerance over that of the newly hatched alevins at comparable acclimation temperatures. The ultimate 7-day TL50 of swim-up alevins was 24.5 C. Swim-up alevins exceed newly hatched alevins in thermal tolerance by 2.0–4.5 C, depending on the temperature of acclimation. The TL50 of newly hatched alevins of comparable acclimation (12 C) is reduced by about 2 C when the exposure time is increased from 1 to 7 days.

McCormick, J. H., K. E. F. Hokanson, and B. R. Jones. 1972. Effects of temperature on growth and survival of young brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Canada 29: 1107–1112.

Nous avons calculé les taux instantanés de croissance et de mortalité et le gain net de biomasse, de l'alevin jusqu'au stade juvénile, d'ombles de fontaine gardés durant 8 semaines à six températures constantes: 7.1, 9.8, 12.4, 15.4, 17.9, et 19.5 C. Le taux de croissance est à son maximum entre 12.4 et 15.4 C. Le taux de mortalité augmente entre 15.4 et 17.9 C, pour atteindre un sommet entre 17.9 et 19.5 C. Le gain net de biomasse est le plus élevé entre 12.4 et 15.4 C.

Nous avons déterminé la médiane des limites de tolérance thermique (valeur TL50) d'alevins nouvellement éclos et d'alevins au stade "swim-up." La tolérance des alevins nouvellement éclos n'augmente pas par suite de l'acclimatation à des températures de 2.5 à 12 C. La température supérieure de TL50 de 7 jours, pour les alevins nouvellement éclos acclimatés à cette gamme de températures, est de 20.1 C. Les alevins au stade "swim-up" font preuve d'une tolérance thermique plus grande lorsque acclimatés à des températures de 7.5 à 12 C. De plus, leur tolérance est supérieure à celle d'alevins nouvellement éclos acclimatés aux mêmes températures. La température supérieure ultime de TL50 de 7 jours, pour les alevins au stade "swim-up", est de 24.5 C. Ces derniers ont une tolérance thermique dépassant de 2.0 à 4.5 C, selon la température d'acclimatation, celle des alevins nouvellement éclos. Lorsque la durée d'exposition est de 7 jours, au lieu de 1, la valeur TL50 d'alevins nouvellement éclos, acclimatés à une même température (12 C), diminue d'environ 2 C.

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This study of the influence of temperature on growth, mortality, net biomass gain, and short-term tolerance in young brook trout has led to a better understanding of the upper temperature requirements of this species during its initial 8 weeks of life. The early period of brook trout life studied in this experiment has been little studied previously and is significant for its many transitions in vital life processes (Mantelman 1960) and its influence on ultimate size of individuals (Myers 1946; Patrick

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and Graf 1962). In addition, the life stage after hatching is most likely to have temperature responses that are different from all other age-groups, given sufficient time for complete acclimation (Fry MS 1951). The greater control of variables and the wider temperature range in our study has enabled us to isolate the temperature effect more precisely than did Haskell et al. (1956).

From accumulated experience with brook trout, usually at different locations with different rearing conditions, fish culturists have derived temperature ranges for successful brook trout culture. Leach (1924) stated that temperatures between 7 and 18 C were the most suitable for brook trout. Davis (1956) narrowed the optimum for growth (age-group unknown) to between 13 and 16 C. Haskell et al. (1956) provided quantitative data on the growth rate of 3.8–12.1-cm brook trout of like origin at different New York State trout hatcheries. Growth was progressively better at each higher temperature over the range of temperatures studied (about 8.0–11.0 C).

Baldwin (1956) studied growth and conversion efficiency of yearling brook trout fed on live minnows at four temperatures: 9, 13, 17, and 21 C. He found maximum growth and food consumption at 13 C and pointed out the higher "cost of living" at 17 C and above. At 17 C the optimum for brook trout activity has been exceeded, whereas that of the minnow has been more closely approached. Graham (1949) found that the scope for activity of yearling brook trout was greatest at 16 C. Fry et al. (1946) established an ultimate upper lethal temperature for young brook trout of 25.3 C, but the newly hatched and first swimming stages were not investigated.

Materials and Methods

The test system provided a continuous flow of water at approximately 250 ml/min at a constant temperature to duplicate test chambers (McCormick and Syrett MS 1970). The glass test chambers were 10 × 10 × 40.5 cm long. The temperatures were monitored with constant-recording telethermometers and were measured once daily in each chamber to the nearest 0.1 C with a mercury thermometer. The mean temperatures reported are the means of the daily thermometer readings except where temperature-control failures occurred. When deviations exceeded 1 C, a daily mean was calculated from the temperature recording chart. The temperatures for duplicate chambers were pooled since they were not significantly different ($P < 0.05$, t-test). Temperatures were maintained within ±0.1 C (95% confidence limits) of the reported means, even though control failures for brief periods permitted temperatures ranging beyond this narrow margin (Table 1). The range of departure from the means and the duration of such departures were not considered sufficient to influence the results attributed to the long-term exposures to the reported mean temperatures.

Test water was Lake Superior water treated with chlorine and ammonia for domestic use in Duluth, Minn. Carbon filtration reduced the chlorine and chloramines to the concentrations reported below. All are below 0.016 mg/liter, the concentration considered safe for chronic exposures of fathead minnows, a fish only slightly less sensitive to chlorine than brook trout (Arthur and Eaton 1971; J. W. Arthur personal communication). Chemical analyses were performed by standard techniques

TABLE 1. Mean instantaneous rates (%/day) of growth, mortality, and net biomass gain of brook trout at constant temperatures for the first 8 weeks after hatching. Values are means of two replicates at each temperature for four sampling periods.

Temperature (C) (mean and range)[a]	Growth (wet weight)	Mortality	Net biomass gain (wet weight)
7.1(5.5–7.9)	2.43	0.18	2.25
9.8(9.5–10.5)	3.83	0.07	3.76
12.4(12.1–16.2)	4.49	0.02	4.47
15.4(15.2–15.9)	4.54	0.02	4.52
17.9(17.7–18.3)[b]	4.01	0.79	3.22
19.5(19.5–19.9)[b]	3.00[c]	5.78[c,d]	−2.78[c]
Tukey's (hsd, $P = 0.05$)	1.05	0.25	1.00

[a]The 95% confidence limits of all reported mean temperatures is <0.1 C.

[b]The data do not include temperatures during the extra day of tempering to these higher temperatures.

[c]Data are computed from only the first 4 weeks data.

[d]Analysis of variance was conducted for temperatures 7.1 through 17.9; mortality at 19.5 C was assumed excessive without analysis.

(Anon. 1965) at six 1-week intervals. A summary of weekly chemical analyses is given below.

	Mean	Range
Total hardness (*mg/liter* as $CaCO_3$)	46.0	45.0–46.0
Calcium hardness (*mg/liter*)	36.0	34.0–41.0
Calcium (*mg/liter*)	14.5	14.1–16.6
Total alkalinity (*mg/liter* as $CaCO_3$)	41.5	41.0–42.0
Total residual chlorine (*mg/liter* as chloramines)	0.007	0.002–0.013
*p*H	7.8	7.7–7.8
Dissolved oxygen (% saturation)	97	92–103

Brook trout were collected in the fall of 1968 from a spawning population indigenous to Mud Creek, Cook County, Minn. The eggs were stripped and fertilized according to usual dry fertilization techniques (Davis 1956). They were incubated at incoming Lake Superior water temperatures, which ranged from 3 to 6 C. Water temperature at hatching was 4.5 C.

Growth at constant temperatures — Test lots of 60 newly hatched alevins were tempered to test temperatures (7.1, 9.8, 12.4, 15.4, 17.9, and 19.5) at a rate approximating 3 C/hr. Growth and mortality were observed over an 8-week period. Those lots assigned to 17.9 and 19.5 C were delayed at 15.4 for 20 hr before transferal to 17.9 C. The lots assigned to 19.5 C were held at 17.9 C for 2 hr before the final transfer. The lots were held in 250-ml beakers of aerated water during tempering to ameliorate the rate of temperature change. The water in the beakers was changed before each transfer.

The fish were fed ad libitum with *Artemia salina* nauplii, supplemented with finely ground beef liver and dry trout starter feed. Rearing units were cleaned daily, and dead fish counted and removed. Subsamples of 10 fish were removed from each test chamber for weight measurements. The sampled fish were not fed on the morning of the sampling day and were held about 6 additional hours without food before they were preserved in formalin-picric-trichloroacetic solution (Davenport 1960). The fish were removed from the preservative, blotted on paper toweling, and weighed intact to the nearest 0.1 mg.

Instantaneous rates of growth (weight), mortality, and net biomass gain were calculated for each sampling interval on a percentage-per-day basis (Ricker 1958). Net biomass gain was derived by subtracting weight lost through mortalities within the lot from weight gained by members of a test lot. Samples were taken at approximately 14-day intervals. The weighted mean rates for the four sampling periods were used as treatment effects in the analysis of treatment differences by one-way analysis of variance and Tukey's multiple range test (Steel and Torrie 1960). Suitable temperature ranges for growth, mortality, and net biomass gain were established within which the departure from the maximum rate of growth, or net biomass gain, or minimum mortality rate, was not greater than Tukey's honestly significant difference (hsd).

Thermal tolerance determination — Alevins reared at, and thus acclimated to, 2.5, 7.0, 7.5, 9.5, 12.0, and 15 C were placed in stainless steel screen containers within test chambers with continuously flowing water for determination of 1-day and 7-day TL50 values. Alevins for these tests were hatched from eggs taken from both the Mud Creek and hatchery-reared adults. At swim-up stage, feeding with brine shrimp was initiated. Sample sizes varied from 10 to 20 alevins per exposure (Table 2). They were transferred from acclimation temperature to test temperatures without tempering. Each day mortalities were recorded, and dead fish and unused food were

TABLE 2. Upper thermal tolerance limits of acclimated brook trout alevins.

No. fish/exposure	Acclimation temperature (to nearest 0.5 C)	Test duration (*days*)	TL50 (*C*)	Confidence limits (95%)	Slope function
		Newly hatched alevin (0.04–0.05 g)			
20	12.0	1	22.5	21.8–23.2	1.03
13	2.5	7	20.4	19.8–21.0	1.06
15	7.0	7	19.4	–[b]	1.17
15	9.5	7	20.4	–[a]	1.02
20	12.0	7	20.2	19.5–20.9	1.05
		Swim-up alevin (0.1–0.2 g)			
10	7.5	7	22.2	–[a]	1.01
10–20	9.5	7	23.7	23.2–24.2	1.01
20	12.0	7	24.8	22.8–26.9	1.09
20	15.0	7	24.3	23.7–24.9	1.05

[a]Plot of two points; no goodness-of-fit test.
[b]The line has a poor fit ($P = 0.05$: chi-square).

removed by pipetting. Percentage mortalities were determined at each temperature and plotted against the exposure temperature on log-probability paper. The TL50, 95% confidence limits, and slope function of the fitted lines were derived by the method of Litchfield and Wilcoxon (1949). The upper 1-day and 7-day TL50 values were determined at two stages of development: one within 24 hr of hatching, and the other at the time of first sustained swimming, henceforth to be referred to as newly hatched and swim-up alevins. The relation between acclimation temperatures and subsequent upper lethal temperatures was evaluated by regression analysis (Steel and Torrie 1960).

Results and Discussion

Mean instantaneous growth rates varied from 2.4%/day at 7.1 C to 4.5%/day at 15.4 C (Table 1, Fig. 1). The temperature range 9.8–17.9 C provided suitable conditions for growth. The maximum rate (4.5%/day) occurred at 12.4 and 15.4 C, but it was not significantly higher ($P < 0.05$, Tukey's test) than the rate at 9.8 C (3.8%/day) or at 17.9 C (4.0%/day). Poorest growth took place at 7.1 (2.4%/day) and 19.5 C (3.0%/day).

Temperatures of low instantaneous mortality rates ranged from 7.1 (0.18%/day) to 15.4 C (0.02%/day). Mortality rates at 17.9 C (0.79%/day) and above were significantly higher ($P > 0.05$, Tukey's test) than the minimum rate at 12.4 and 15.4 C (0.02%/day).

Instantaneous rates of net biomass gain were highest between 9.8 (3.8%/day) and 15.4 C (4.5%/day). The maximum occurred at 12.4 and 15.4 C (4.5%/day). The poorest net biomass gain occurred at 19.5 C (–2.78%/day), where weight loss through mortality exceeded weight gain by survivors. Rates at 7.1 (2.3%/day) and 17.9 C (3.2%/day) were intermediate in magnitude but significantly below the maximum rate ($P > 0.05$, Tukey's test).

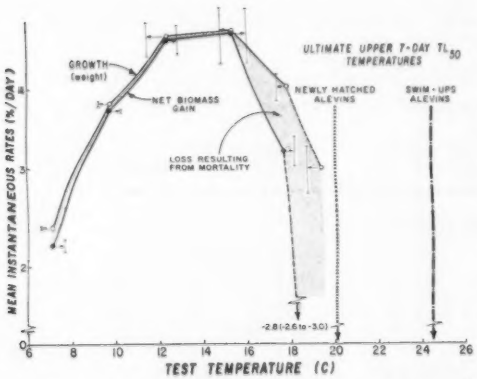

FIG. 1. Effects of temperature on mean instantaneous rates of growth, mortality, net biomass gain, and ultimate 7-day median temperature tolerance limits of brook trout during their first 8 weeks after hatching. Vertical bars indicate the range of the replicate means. Dashed lines indicate data obtained from only the first 4 weeks of temperature exposure.

At 7.1 C, 50% of the newly hatched alevins reached swim-up stage in 56 days. At 9.8–19.5 C, this transition required only 19–27 days. The time required to make the transition from newly hatched to swim-up stage is ecologically significant, because the alevins are especially vulnerable to predation by fish (Ricker 1941; Herting and Witt 1967) and aquatic insects (Claire and Phillips 1968; A. V. Nebeker personal communication) between emergence from the redd and swim-up stages. It is therefore believed that it would be advantageous to bridge this period as quickly as is physiologically permissible.

The TL50 values for brook trout alevins vary with age (Table 2). Newly hatched alevins were more sensitive to high temperature than swim-up alevins acclimated to similar temperatures. Newly hatched alevins acclimated to 7.0 C had a 7-day TL50 of 19.4 C; the 7-day TL50 of swim-up alevins acclimated to 7.5 C was 22.2 C. Newly hatched alevins acclimated to 12 C had a TL50 of 20.2, and swim-up alevins, 24.3 C. Consistent with previous findings, duration of exposure to test temperature may also alter TL50 temperatures. At the newly hatched stage, alevins of comparable acclimation (12 C) have a 1-day TL50 of 22.5 C; after a 7-day exposure the TL50 is reduced to 20.2 C.

The 7-day TL50 temperature of swim-up alevins increased at a rate of 0.6 C for each degree increase in acclimation temperature over the range 7.5–12 C. The formula $Y = 18.04 + 0.57\,X$ describes the line that best fits these data, where Y is the lethal temperature and X the acclimation temperature. The coefficient of determination ($r^2 = 0.98$) for the fit of this line to the data indicates that only 2% of the variability in TL50 cannot be ascribed to acclimation temperature. At 15 C acclimation no additional increase in lethal temperatures was observed. This failure to show an increase in tolerance indicates that between 12 and 15 C the ultimate thermal acclimation for this life stage is reached. The ultimate 7-day TL50 temperature of swim-up brook trout is thus estimated to be 24.5 C (midway between the TL50 values for 12 and 15 C acclimation). This temperature is in basic agreement with the 25.3 C reported by Fry et al. (1946) for yearling brook trout. However, the ultimate 7-day TL50 tem-

perature for newly hatched alevins is 20.1 C, considerably lower than for the older fish studies by Fry et al. (1946). Newly hatched alevins fail to exhibit a relation between acclimation temperature and thermal tolerance, perhaps because the physiological mechanism responsible for acclimation has not yet been adequately developed. Bishai (1960), working with 30-day-old *Salmo salar*, *S. trutta*, and *S. trutta fario* reared at 5–6 C, and thus pre-swim-up in development, also found little or no acclimation influence on subsequent 7-day TL50 determinations. An increase in acclimation from 5 to 6 C (7-day TL50, 22 to 22.5 C) to 10 and 20 C increased the 7-day TL50 values to only 23 C, for both the 10 and 20 C acclimations. Mantelman (1960), also working with early developmental stages, reports that rainbow trout before swim-up fail to move in a temperature-selective manner in a thermal gradient as they do after swim-up.

This study indicates that a suitable range of temperatures for growth and survival of alevin through juvenile brook trout is from 9.8 to 15.4 C, that the optimum lies between 12.4 and 15.4 C when food is not a limiting factor, and that temperatures of 18 C or above would be detrimental except for periods of short duration. The daytime temperature, 19 C, associated with the downstream limit of brook trout distribution (Burton and Odum 1945) closely approximates the 17.9 C found to limit net biomass gain in this study. Temperatures at or slightly above 20 C may be tolerated for short periods by newly hatched alevins, or at or slightly above 25 C by swim-up alevins, but at such temperatures mortalities can be expected to be severe if exposure is extended.

Several field studies (Creaser 1927, 1930; Burton and Odum 1945; Vincent and Miller 1969) indicate that brook trout populations depend upon their ability to survive at the extremes of their thermal limits, and that they prosper within the relatively narrow limits suitable for their sustained growth and survival, yet cool enough to limit competitors. Where competitors are limited by some factor other than temperature and available food becomes less limiting to the brook trout, they will be able to prosper at temperatures more closely approaching the upper thermal limits for maximum growth. However, where food is limiting, more efficient use of available food will be made at temperatures cooler than those promoting maximum growth on unlimited rations (Brett et al. 1969).

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Use of Otoliths in Identifying Herring Stocks in the Southern Gulf of St. Lawrence and Adjacent Waters[1]

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MESSIEH, S. N. 1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. J. Fish. Res. Bd. Canada 29: 1113–1118.

Examination of 1423 herring from various fisheries in the southern Gulf of St. Lawrence and adjacent waters indicates that otoliths can be used as "natural tags" for identifying herring stocks. Two types of otoliths are described: an S-type characterizing spring-spawning herring and an A-type characterizing autumn-spawning herring. Over 97% of herring from spring and autumn fisheries had otoliths of the respective types. There appeared to be sub-divisions within each type.

The distinguishing of these otolith types provides evidence that spring- and autumn-spawning herring mix on American Bank and off Shippegan in the Gulf of St. Lawrence and in Chedabucto Bay. The validity of otolith types as criteria for separating herring stocks was supported by comparison with relations of otolith length to fish length and with fin ray counts.

MESSIEH, S. N. 1972. Use of otoliths in identifying herring stocks in the southern Gulf of St. Lawrence and adjacent waters. J. Fish. Res. Bd. Canada 29: 1113–1118.

L'examen de 1423 harengs provenant du secteur méridional du golfe Saint-Laurent et des eaux avoisinantes démontre qu'il est possible d'utiliser les otolithes comme "marques naturelles" pour l'identification des stocks. L'auteur décrit deux types d'otolithes: le type S, caractéristique des harengs frayant au printemps et le type A, appartenant aux harengs qui fraient en automne. Plus de 97% des harengs capturés au printemps et en automne possèdent des otolithes du type qui leur est propre. Il semble y avoir subdivisions au sein de chacun des types.

Ces types d'otolithes démontrent que le hareng frayant au printemps se mélange, sur le banc des Américains et au large de l'île Shippegan dans le golfe Saint-Laurent et dans la baie Chedabucto, avec celui qui fraie en automne. Les relations entre la longueur de l'otolithe et la longueur du poisson et le nombre de rayons des nageoires établissent la validité des types d'otolithes pour l'identification des stocks de harengs.

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IN this report, the various shapes and structures of otoliths are used to distinguish herring stocks in the southern Gulf of St. Lawrence. Though, other workers (Einarsson 1951; Parrish and Sharman 1958, 1959; Raitt 1961) have used otoliths to identify herring groups in the northwest Atlantic they used otolith characteristics (optical appearance of nucleus, relative sizes of growth zones and winter rings) that could vary with environmental changes (Messieh 1969). The characters used here are less affected by the environment and hence, seem more reliable in distinguishing stocks.

Materials and Methods

Otoliths from 14 samples totaling 1423 herring were examined. Samples were collected from various localities (Fig. 1) during 1968 through 1970. A total of 62 otoliths were discarded (Table 1) because they were broken or badly mounted or showed irregularities (such as translucence; differences within pairs).

Otoliths were examined in a dry condition under magnification (×12) using reflected light. Preliminary examination showed that the two otoliths in each pair had similar shapes and one was almost a mirror image of the other. Hence, the right otolith was selected for measurements.

The nuclei (Fig. 2) were measured with a moving ocular micrometer scaled in equal divisions (one unit = .083 mm under magnification ×12). Where the nuclei were not circular, diameters were taken as averages of the long and short axes. Diameters of otolith nuclei were plotted as frequency distributions and compared by chi-square tests for homogeneity.

Images of all otoliths were traced using microscope camera lucida. Their lengths (distances between tips of rostrum and pararostrum) and angles (between lines joining rostrum, postrostrum, and pararostrum) were measured. Ratios of postrostra to pararostra widths were calculated.

[1]Canadian Contribution to the International Biological Program No. CCIBP-150.

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Stages of maturation were recorded according to the maturity scale adopted by the Herring Committee of the International Council for the Exploration of the Sea (Anon. 1962).

Except for four samples taken in 1968, dorsal and pectoral fin rays were counted from radiographs. In 1968 samples of fin rays were counted directly from the fresh material. Differences between the means of fin ray counts were compared by *z*-test.

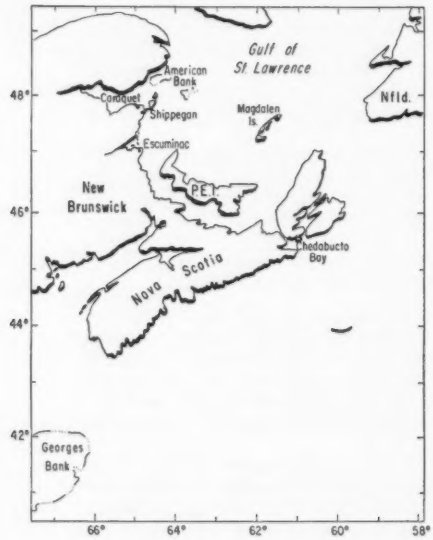

FIG. 1. Localities where herring samples were collected.

Types of Otoliths and Useful Characters

According to their shape, otoliths were classified into two types: the spring-type (S) and the autumn-type (A) (Fig. 3).

S-type — These have well-developed pararostra (Fig. 4A–C) which in most cases are as long as the postrostra. The excisura minor is deeply grooved, clearly defined, and almost parallel to the long axis of the otolith. The line joining the tips of the postrostrum and pararostrum runs almost perpendicular (angle ranging from 84.4 to 86.2°) to the long axis of the otolith (Table 2).

Within this type, otoliths from different areas showed minor differences. For example, samples from Magdalen Islands were more slender than those from Caraquet (Fig, 4A–C).

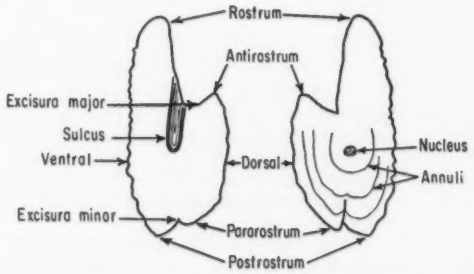

FIG. 2. Otolith of Atlantic herring showing different parts from the proximal (*left*) and distal (*right*) views. Drawing to scale. ×12.

TABLE 1. Numbers of fish used for otolith measurements, maturation stages, and fin ray counts.

Sampling locality	Date	No. of fish in sample	Otolith measurements and maturation	Pectoral fin ray counts	Dorsal fin ray counts
Caraquet	May 1968	150	148	124	124
Magdalen Is.	May 1968	93	85	85	85
Caraquet	August 1968	150	141	141	141
Escuminac	August 1968	100	96	–	–
American Bank	October 1968	50	42	42	42
American Bank	June 1969	100	90	90	90
Off Shippegan	September 1969	80	75	75	75
Chedabucto Bay	February 1969	100	93	93	93
Chedabucto Bay	April 1969	100	94	–	–
Caraquet	May 1970	100	100	100	99
Magdalen Is.	May 1970	100	100	100	100
Georges Bank	July 1970	100	97[a]	97	97
Caraquet	September 1970	100	100	99	98
Escuminac	September 1970	100	100	100	99
Total		1423	1361	1146	1143

[a]For otolith measurements only.

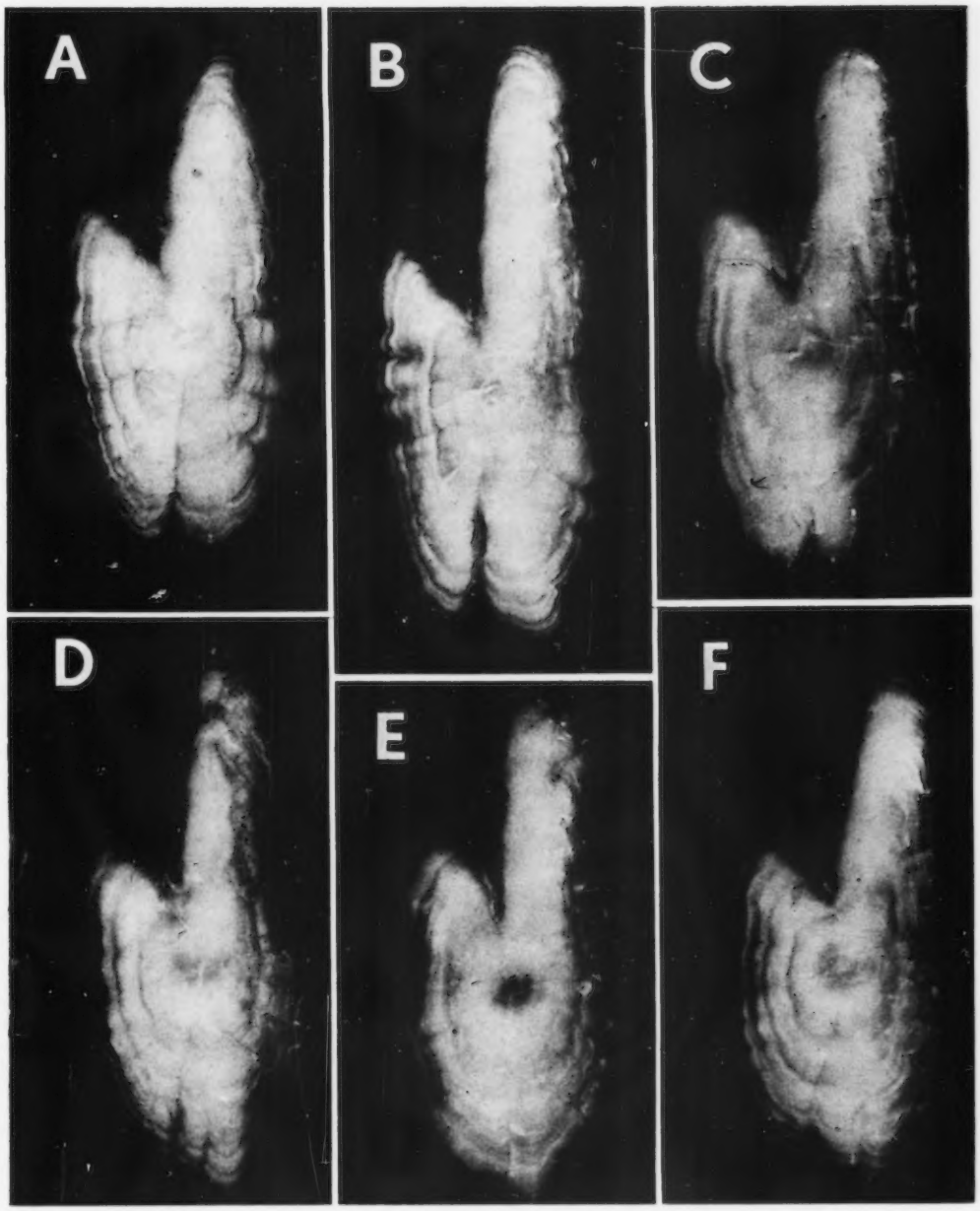

FIG. 3. Herring otoliths from various fisheries showing S-type (A–C) and A-type (D–F). A, Caraquet, May 1968; B, Magdalen Is., April 1970; C, Chedabucto Bay, April 1970; D, Caraquet, September 1970; E, Georges Bank, July 1970; F, Chedabucto Bay, April 1970.

Messieh — J. Fish. Res. Bd. Canada

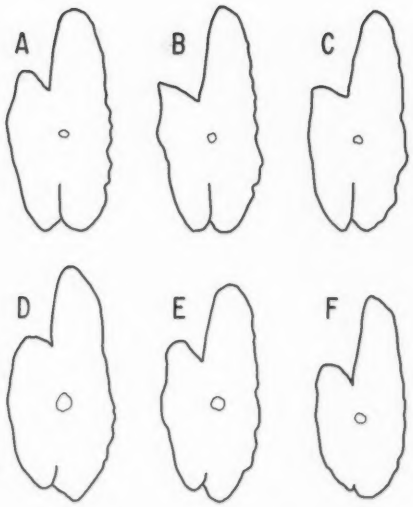

FIG. 4. The two types of otoliths from spring and autumn fisheries; A-C, S-type; D-F, A-type. A, Caraquet, May 1968; B, Magdalen Is., May 1968; C, Magdalen Is., April 1970; D, Caraquet, August 1968; E, Escuminac, August 1968; F, Georges Bank, July 1970.

TABLE 2. Mean values of otolith measurements from the various fisheries sampled (Nucleus diameter in ocular micrometer units 1 = 0.083 mm).

Locality	Date	Mean diameter of nucleus	Ratio of postrostrum/pararostrum width	Angle between lines from rostrum to postrostrum and pararostrum
		Spring fishery		
Caraquet	May 1968	3.08±1.28[a]	1.39±.36	84.4±5.88
Magdalen Is.	May 1968	3.40±1.02	1.37±.30	85.3±5.72
Caraquet	May 1970	3.12±.98	1.32±.33	86.2±5.34
Magdalen Is.	May 1970	3.36±1.10	1.42±.31	84.5±5.82
		Autumn fishery		
Caraquet	Aug. 1968	4.06±1.25	2.80±.84	68.8±6.11
Escuminac	Aug. 1968	4.22±1.32	3.12±.96	65.7±5.90
Georges Bank	July 1970	4.27±1.09	3.95±1.45	66.9±5.35
Caraquet	Sept. 1970	4.21±1.15	3.10±.92	65.3±5.44
Escuminac	Sept. 1970	4.15±1.21	3.45±1.05	
		Mixed fishery		
American Bank	Oct. 1968	3.86±1.13	1.95±.60	78.2±5.93
American Bank	June 1969	3.52±.92	2.85±.73	73.0±6.17
Off Shippegan	Sept. 1969	3.74±1.02	2.75±.98	70.2±5.61
Chedabucto Bay	Feb. 1970	3.70±1.25	2.93±1.01	74.9±5.83
Chedabucto Bay	Apr. 1970	3.62±.94	2.45±.95	71.4±6.15

[a]One standard deviation.

In the spring fishery (Table 3), S-type otoliths were predominant (97.7%).

A-type — Otoliths of this type have small pararostra that are always shorter than the postrostra (Fig. 4D–F). The excisura minor is not deeply grooved. The postrostrum is more than twice as wide as the pararostrum. A line extending from the nucleus to the excisura minor usually forms an angle with the long axis of the otolith. The line joining the tips of the postrostrum and pararostrum is obviously not perpendicular (angle ranging from 65.3 to 68.8°) to the long axis of the otolith.

Within this type there were also minor differences between areas. For example, otoliths from Georges Bank (Fig. 4F) had poorly developed pararostra, which obviously differed from those of the Gulf of St. Lawrence (Fig. 4 D,E).

In the autumn fishery (Table 3), 97.1% of the herring had A-type otoliths.

Mixed types — Samples of otoliths from American Bank and offshore from Shippegan (Fig. 5A) showed a mixture of S- and A-types. Similarly, samples from Chedabucto Bay (Fig. 5B) showed a mixture of the two types. When herring from these fisheries

TABLE 3. Otolith types from the various fisheries and maturation stages of herring (S = Spring-type otolith; A = Autumn-type; D = Doubtful).

Locality	Date	Type of otolith	No. of fish	Maturation stages[a] 2	3	4	5	6	7	8
			Spring fishery							
Caraquet	May 1968	S	148			2	11	120	10	5
		D	2							
Magdalen Is.	May 1968	S	85			12	37	36		
		D	8							
Caraquet	May 1970	S	100				9	85	6	
		D	0							
Magdalen Is.	May 1970	S	100			3	8	77	12	
		D	0							
			Autumn fishery							
Caraquet	Aug. 1968	A	141		6	2	25	69	34	5
		D	9							
Escuminac	Aug. 1968	A	96				14	82		
		D	4							
Caraquet	Sept. 1970	A	100				7	85	8	
		D	0							
Escuminac	Sept. 1970	A	100				8	67	22	3
		D	0							
			Mixed fishery							
American Bank	Oct. 1968	S	22	1	13	6				2
		A	20	3	2	3				12
		D	8							
American Bank	June 1969	S	24	1	5	11	1		1	5
		A	66	4	10	46	6			
		D	10							
Off Shippegan	Sept. 1969	S	18	12	1				5	
		A	57	14	2		1		37	3
		D	5							
Chedabucto Bay	Feb. 1970	S	20	6	9	5				
		A	73	25	36	7				5
		D	7							
Chedabucto Bay	Apr. 1970	S	26	2	8	16				
		A	68	20	42	6				
		D	6							

[a]Stages 5, 6, and 7 are prespawning, spawning, and postspawning fish. Others are maturing or recovering.

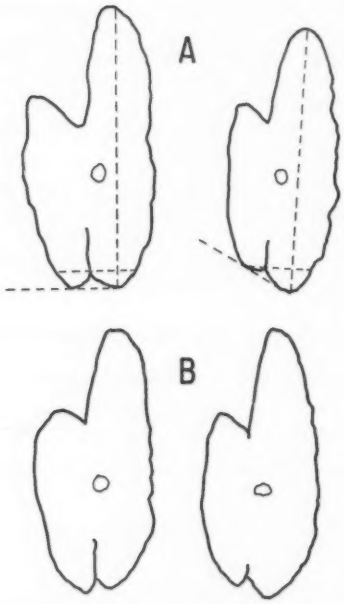

FIG. 5. Otoliths from the mixed fisheries showing S-type (*left*) and A-type (*right*) from: A, American Bank, June 1969; B, Chedabucto Bay, April 1970.

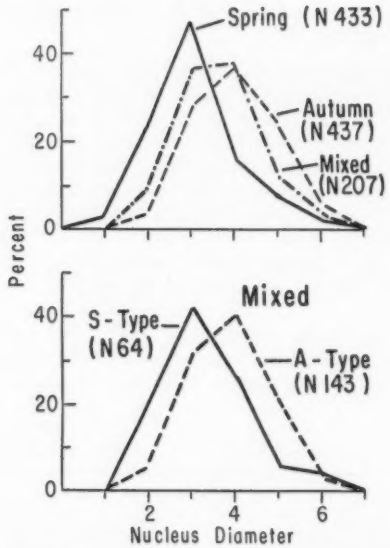

FIG. 6. Frequency distributions of otolith nuclei diameters (1 unit = 0.083 mm) of herring from three fisheries in southern Gulf of St. Lawrence (*upper*) and from the mixed fishery after separation by the two otolith types (*lower*).

were separated by otolith types (Table 3), the maturation stages of each type indicated that the fish belonged to the same spawning group.

Nucleus diameter — Mean diameters of nuclei from spring herring were less than those from autumn herring (Table 2), and the differences in all cases were significant ($P = 0.05$).

When the nucleus diameters were plotted as frequency distributions, there was overlapping between otoliths from different fisheries (Fig. 6). However, the differences between them were significant. The probability that spring and autumn fish in the Gulf of St. Lawrence were drawn from the same population was not significant ($P < .01$). Similarly, probabilities that fish from American Bank and offshore from Shippegan were from the same populations as the other spring and autumn fisheries were not significant ($P < .01$).

When otoliths from mixed areas were separated by type, the frequency distributions of their nucleus diameters were found similar to those of the spring and autumn fish in the Gulf of St. Lawrence (Fig. 6). The probability was .25 that A-type and S-type otoliths were drawn from the autumn- and spring-spawning populations, respectively ($\chi^2 = 9.47$, with 7 degrees of freedom and 8.17, with 6 degrees of freedom, respectively).

Supporting Evidence of Usefulness

Relation of otolith length to fish length — The regression of otolith lengths on fish lengths differed between otolith types and localities (e.g. Fig. 7A–C). When samples from mixed fisheries (e.g. Fig. 7D) were separated by otolith types, the regressions of otolith lengths on fish lengths between the two types were significantly different ($P > .01$).

Fin ray counts of S- and A-type herring — Mean counts of pectoral and dorsal fin rays (Table 4) differed significantly ($P > .01$) between herring of spring and autumn fisheries and between herring of spring S- and A-type otoliths. In all cases mean fin ray counts were smaller for S-type otoliths. However, the differences were more obvious for pectoral than for dorsal fins.

In samples where both otolith types were mixed, mean values of fin ray counts were intermediate between those of S- and A-types. When these mixed fish were separated according to otolith types, the fin ray counts differed between types, the mean counts being less for S-type than for A-type.

Discussion and Conclusions

The evidence presented indicates that otoliths can be used as "natural tags" for identifying herring stocks in the study area. The differences between the two types are clear-cut, and hence, can be used for identifying the stock with the aid of a few specimens. The characteristics can be determined easily even in the field, as part of the routine examination of otoliths for aging purposes.

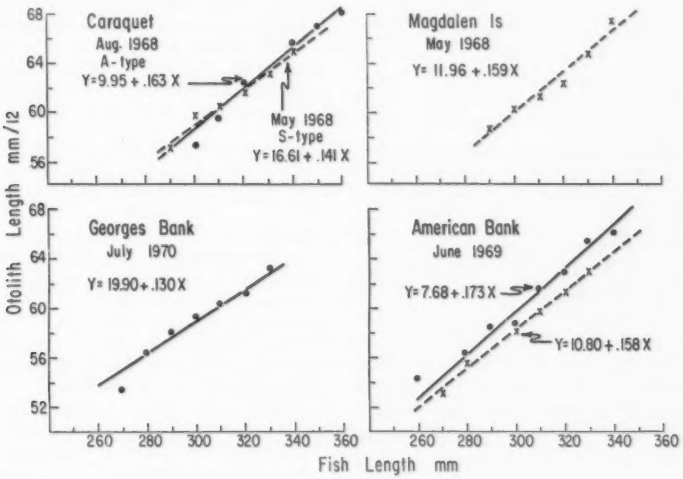

FIG. 7. Regressions of otolith lengths on fish lengths for herring from: spring fisheries in Caraquet and Magdalen Is., autumn fisheries in Caraquet and Georges Bank, and mixed fishery on the American Bank.

TABLE 4. Mean pectoral and dorsal fin-ray counts of herring from various fisheries, separated by otolith types. One standard deviation of mean and number of fish (*in parentheses*) included.

Locality	Date	Pectoral fin		Dorsal fin	
		S-type otolith	A-type	S-type	A-type
		Spring fishery			
Caraquet	May 1968	17.04±.70(124)		19.36±.78(124)	
Magdalen Is.	May 1968	17.18±.61(85)		19.23±.65(85)	
Caraquet	May 1970	17.16±.65(100)		19.36±.72(99)	
Magdalen Is.	May 1970	17.22±.68(100)		19.28±.67(100)	
		Autumn fishery			
Caraquet	Aug. 1968		18.31±.64(141)		19.45±.63(141)
Georges Bank	July 1970		18.02±.78(97)		19.30±.72(97)
Caraquet	Sept. 1970		18.55±.77(99)		19.51±.61(98)
Escuminac	Sept. 1970		18.49±.84(100)		19.38±.60(99)
		Mixed fishery			
American Bank	Oct. 1968	17.35±.65(22)	18.23±.62(20)	19.06±.78(22)	19.21±.75(20)
American Bank	June 1969	17.76±.85(24)	18.31±.68(66)	18.75±.80(24)	19.00±.82(66)
Off Shippegan	Sept. 1969	17.70±.82(18)	18.00±.75(57)	19.11±.69(18)	19.34±.72(57)
Chedabucto Bay	Feb. 1970	17.55±.84(20)	17.96±.81(73)	18.45±.86(20)	18.56±.80(73)

Although the present data do not demonstrate the genotypic origin of the differences in otolith characteristics, the consistency of differences between the two types, in conjunction with differences in other characteristics, suggests that the two otolith types represent fish of biologically isolated herring populations.

That the nuclei diameters were less for the S-type than for the A-type can be explained by the fact that spring-hatched larvae have a shorter larval period than autumn-hatched larvae (Jean 1956).

The differences in regressions of otolith lengths on fish lengths between types and localities suggest that each type represents more than one stock. This is also supported by the minor differences observed within each otolith type.

No samples of smaller herring were available to determine whether or not otoliths grew differently before the fish attained sexual maturity. Watson (1964) plotted the regression of otolith lengths on fish lengths for young herring in the Gulf of Maine, but he did not compare fish from different areas.

Differences between fin ray counts of herring from the Gulf of St. Lawrence, Nova Scotia, and Georges Bank indicate that S-type herring were hatched in the spring (conditions of rising temperature, and hence smaller fin ray counts) and those of A-type were hatched in the autumn (conditions of falling temperature, and hence larger fin ray counts). These results coincide with results obtained from fin ray counts of spring- and autumn-spawning herring in southern Gulf of St. Lawrence (Messieh and Tibbo 1971) and off southwestern Newfoundland (Parsons and Hodder 1971)

Advantages of the use of otolith structure for identifying herring stocks, as compared with meristic method, lie in the fact that fish can be separated individually. Thus, the method offers promise in studying interrelations between stocks and estimating their mixing rates.

Acknowledgments

I wish to express my thanks to Messrs C. F. Monaghan and W. H. Dougherty who made most of the field collections. Mr C. A. Dickson prepared camera lucida drawings of the otoliths. Thanks are extended also to Dr J. S. Scott and Mr S. N. Tibbo for constructive criticism of the manuscript.

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Effect of Intertidal Exposure on Survival and Embryonic Development of Pacific Herring Spawn[1]

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JONES, B. C. 1972. Effect of intertidal exposure on survival and embryonic development of Pacific herring spawn. J. Fish. Res. Bd. Canada 29: 1119–1124.

Eggs of Pacific herring were exposed to air for different periods of time to simulate tidal effects on spawn deposits at varying beach heights on the British Columbia coast. Prehatching mortality changed from 13% unexposed to 31% at 8-hr exposure twice daily. Significantly higher mortality occurred among eggs of smaller fish and eggs in smaller clumps. Incubation time decreased from 19.2 to 18.2 days with 2-hr exposure twice per day, and thereafter declined 0.4 days with greatest exposure. Larval length at hatching for unexposed eggs was 7.7 mm; lengths for all degrees ot exposure were similar (7.2 mm). Larval weight was greatest (0.099 mg) for eggs exposed between 4 and 6 hr twice per day, decreasing with either greater submergence or exposure (7 and 12%, respectively). Hypoxia, desiccation, and the air-water temperature differential are considered as possible causal agents. A field survey indicated that deposition of larger eggs occurred at upper beach levels. These results suggest that a high fishing intensity could detrimentally affect future spawn and thereby reduce population recruitment.

JONES, B. C. 1972. Effect of intertidal exposure on survival and embryonic development of Pacific herring spawn. J. Fish. Res. Bd. Canada 29: 1119–1124.

L'auteur a exposé à l'air des oeufs de hareng du Pacifique durant des périodes de temps plus ou moins longues, en vue de simuler l'action de la marée sur le frai déposé à différents niveaux sur les plages de la Colombie-Britannique. Les mortalités précédant l'éclosion sont passées de 13% pour des oeufs non exposés à 31% pour des oeufs exposés durant une période de 8 heures, deux fois par jour. Les oeufs de petits poissons et les oeufs en petites masses ont subi des mortalités nettement plus élevées que les autres. La période d'incubation est passée de 19.2 à 18.2 jours après expositions de 2 heures, deux fois par jour, pour diminuer ensuite de 0.4 jour après l'exposition la plus longue. Les larves provenant d'oeufs non exposés mesuraient 7.7 mm à l'éclosion. Dans le cas d'oeufs exposés, les larves étaient de même longueur (7.2 mm) à toutes les durées d'exposition. Le poids le plus élevé (0.099 mg) a été observé chez les larves provenant d'oeufs exposés durant 4 à 6 heures, deux fois par jour. Une plus longue période de submergence ou d'exposition a causé une diminution de poids (7 et 12% respectivement). L'auteur considère l'hypoxémie, la dessication et le différentiel thermique air-eau comme causes possibles de ces changements. Un relevé sur le terrain démontre que les plus gros oeufs se trouvent à des niveaux plus élevés sur la plage. Il en conclut qu'une haute intensité de pêche pourrait nuire au frai subséquent, réduisant ainsi le nombre de recrues se joignant à la population.

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EGGS of Pacific herring (*Clupea pallasii*) are spawned in and below the intertidal zone where they adhere to seaweed. Those deposited higher in the zone are subjected to increased regular tidal periods of exposure to air; effects of exposure could thus be related to beach height. Owing to adhesiveness and repetitive spawning, egg clumps of differing thickness are formed which possibly alter these effects. Variation in individual spawner size results in eggs of different sizes that might also be influential.

Fishing intensity can reduce mean size and numbers of herring spawners. Where this occurs, mean egg and clump size may also decrease. Recruitment potential could therefore be decreased if environmental factors operating in the intertidal zone are more detrimental to smaller eggs, eggs from smaller fish, or smaller clump sizes.

Previous laboratory work on herring egg development has been concerned with conditions for submerged eggs. This study sought to examine

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science from the University of British Columbia.

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prehatching mortality, incubation time, and larval length and weight at hatching in relation to varying degrees of exposure, using as additional variables effects of egg size (dry weight), fish size, and clump size. Beach surveys were also undertaken to note possible egg size stratification.

Materials and Methods

Spawner Characteristics Analysis

Eggs were obtained from 33 spawning Pacific herring (lower east coast stock of B.C.) for the laboratory work. Gonad remains were preserved in 5% formalin. Standard length and wet weight (minus gonads) were recorded for each fish; age was determined from scale analysis (Tester 1937) and checked against otolith readings. Five lots of 100 eggs were subsampled from the preserved gonads of each fish, and oven dried for 24 hr at 50 C (Blaxter and Hempel 1963), then weighed to the nearest 0.1 mg.

Laboratory Exposure Experiment

Five tanks, each containing 33 incubators simulated conditions at different beach heights (Fig. 1) ranging from the control (0) which was continuously submerged, through a 2-, 4-, 6-, and 8-hr exposure twice per day. From every spawner five batches of eggs were stripped, each into a separate incubator (mean egg count ± 1 SD = 132 ± 43). The eggs were then fertilized, flushed with fresh sea water, and transferred to their respective exposure tanks where they remained submerged for 12 hr before the experiment began.

The artificial environment approximated that recorded on beach spawning sites. One daily exposure period was in the light (day length = 13 hr) and the other in darkness. Eggs were submerged in running sea water (mean flow rate = 11 ml/cm²/min) at 5 cm depth. Salinity was close to 30.0‰ at a temperature of 8 C and oxygen levels near 6.5 ml/liter. When exposed, eggs were subjected to an air temperature of 12 C with 65% relative humidity.

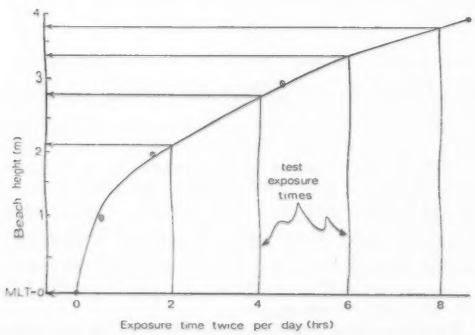

FIG. 1. Relation of beach height to exposure time (Vancouver, B.C.; March 1970). MLT = mean low tide.

After 15 days larvae began to hatch, and were collected immediately before each exposure period. Upon removal they were immobilized in a 1:50,000 solution of MS 222 and preserved in 5% formalin. When larval emergence ceased, dead eggs in each incubator were counted. From these data prehatching mortality and incubation time (from fertilization to 50% hatch) were determined. Preserved larvae were counted and total lengths of all measurable larvae determined with a micrometer eyepiece. Mean length of larvae from each incubator was proportionally corrected for a shrinkage of 4.2% which occurred during the 3-month period of preservation. Preserved larvae were washed in fresh water, oven dried at 50 C for 24 hr, and weighed. Mean larval weight so determined equals total weight of body and yolk sac.

Egg Size Distribution Beach Surveys

From each of three spawning sites five egg samples were collected, ranging from as low as practicable to the location of the highest deposit, and preserved in 5% formalin. Sample beach-height relative to mean low tide was determined from the datum established by sea level at the exact time of low tide. Preserved eggs were separated from seaweed by washing, immersing in KOH (1N) solution, heating to 30 C, and allowing to stand for 2 hr. Each sample was transferred to 5% formalin for 24 hr to harden eggs before adhering seaweed was removed. Eggs were then washed in fresh water, and 10 100-egg subsamples were taken from each beach level to determine mean dry weight.

Results

Initially, the data were analysed in total. They were then ranked for each of the additional variables (egg size, as determined from preserved gonads; fish length; and clump size, as a function of egg number), subdivided into three groups, and treated again. Actual values (variable mean ± 1 SD) for these groupings were:

Additional Variables	Grouping		
	Small	Medium	Large
Egg size (*mg*)	0.243±0.015	0.271±0.005	0.300±0.015
Fish length (*mm*)	199±5	211±4	223±6
Clump size (*no.*)	89±17	130±12	175±29

The possible influence of fish age and weight were not examined as spawners were 73% 3-year-olds,

and their lengths and weights were highly correlated ($R = 0.95$).

Prehatching Mortality

Prehatching mortality increased continuously with increased exposure time (Fig. 2A), rising from 13% in the control to 31% in the 8-hr exposure period ($P < .01$). Eggs from smaller fish had a higher mortality (Fig. 2B) than larger fish, but the effect was not significant ($.05 < P < .10$). Smaller egg clumps had significantly higher mortality ($P < .01$). than larger egg clumps (Fig. 2C).

Incubation Time

Relation of incubation time to exposure time is shown in Fig. 3. Eggs in the control had an incubation period of 19.2 days, but first exposure produced an abrupt decrease of 1 day ($P < .01$). Thereafter, there was only a gradual decrease of 0.4 days with increased exposure time ($.01 < P < .05$).

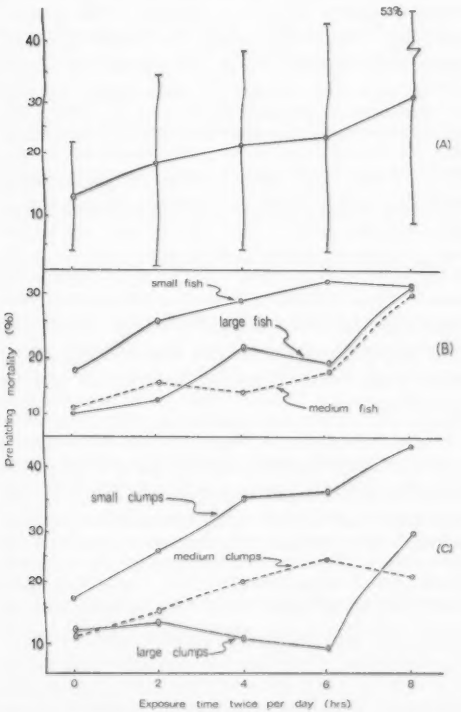

FIG. 2. Relation of mean prehatching mortality to exposure time for (A) total data (± 1 SD), (B) fish length effects, and (C) clump size effects.

Larval Length

Larval length at hatching in relation to exposure time (Fig. 4A) decreased from 7.7 mm in the control to 7.2 mm with a 2-hr exposure twice daily ($P < .01$). Increased exposure produced no further decrease in length. Larvae from smaller eggs were shorter (Fig. 4B), but this difference was not significant ($.05 < P < .10$).

Larval Weight

Relation of larval weight at hatching to exposure time reached a maximum (0.099 mg) with

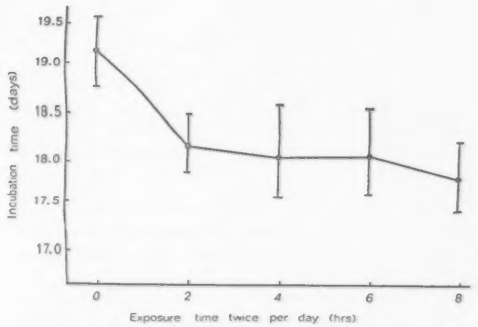

FIG. 3. Relation of mean incubation time (± 1 SD) to exposure time for total data.

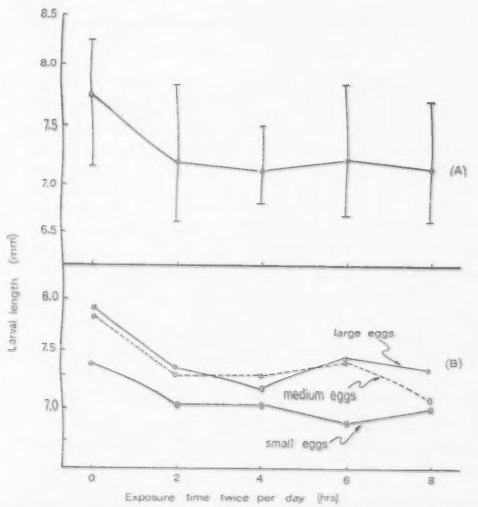

FIG. 4. Relation of mean larval length to exposure time for (A) total data (± 1 SD) and (B) egg size effects.

exposure periods of 4–6 hr (Fig. 5A). However, differences were not significant. Egg size had a pronounced effect ($P < .01$) on larval weight (Fig. 5B).

Beach Stratification

A beach survey at the time of spawning (Fig. 6) showed an increase in egg weight with beach height ($P < .01$). Surveys taken later in the incubation period indicated that this relation had disappeared.

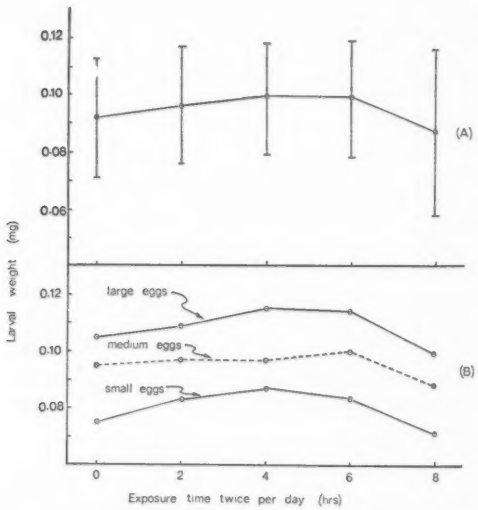

Fig. 5. Relation of mean larval dry weight to exposure time for (A) total data (± 1 SD) and (B) egg size effects.

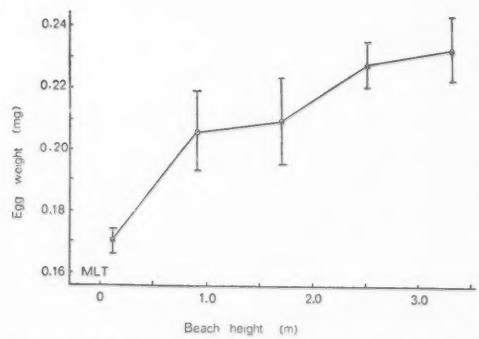

Fig. 6. Relation of mean egg dry weight to beach height at spawning (Bedwell Bay; April 20 1970). MLT = mean low tide.

Discussion

The salient features of this study suggest that hypoxia, desiccation, and the air-water temperature differential are primary factors affecting exposed herring spawn, although egg size and number per clump appear to exert an influence upon their effects.

In terms of prehatching mortality, combined effects of hypoxia and desiccation seem most important, and possibly become more severe with increased exposure. Egg density seems to produce a compromise between them. Thus, as clump size gets larger, volume increases at a faster rate than surface area, thereby reducing the proportion of surface eggs. This exposes a smaller proportion of the total eggs in larger clumps to effects of desiccation, but a greater proportion to potential hazards of low oxygen delivery. An optimum clump size is therefore expected. Although smaller clumps incurred higher mortality, a similar situation was not found in larger clumps. Perhaps larger clumps were not dense enough for effects of hypoxia to cause a higher mortality among interior eggs. Optimum clump size was approximately seven eggs thick, roughly twice that found by Taylor (1971) who, for submerged eggs only, reported an optimum layer density of two to four eggs. The discrepancy may be related to his water flow rate which was less than half that used in this study. Thus, lower oxygen levels or accumulated inhibitory waste could have decreased his egg density optimum.

The same volume to surface area relation applies to eggs of different sizes. Thus, smaller eggs which have a proportionally greater surface area than larger eggs could be most affected by desiccation. This may account for higher egg mortality from smaller fish, since smaller fish produce smaller eggs (Blaxter and Hempel 1963). It is also reported (Toom 1958) that less viable embryos are produced by smaller fish. Since there is a twofold increase in oxygen requirements over the incubation period (Volodin 1956), higher mortality could thus stem from introducing such embryos to lower oxygen regimes when near hatching and their oxygen needs are maximal. The exposed membrane could prevent hatching and cause suffocation as available oxygen is no longer sufficient to cover minimum needs.

Since air and sea water temperatures are commonly unequal, a difference in exposure time would alter the proportion of incubation time during which eggs would be subjected to either temperature regime. If air temperature is higher than sea water, mean incubation temperature will increase with greater exposure time. This could increase

the rate of desiccation and possibly produce a higher prehatching mortality.

Increased incubation temperature could also result in a higher rate of metabolism. Resultant faster-growing embryos could thus reach the hatching stage more rapidly. Incubation time might also decrease if eggs under hypoxial stress tend to hatch prematurely (Rannak 1958). Thus, when embryos begin development oxygen needs are possibly satisfied when exposed and submerged, but later when demand is much higher, the exposed egg membrane may decrease available oxygen and initiate hatching. Since increased temperature is possibly related to available oxygen, these phenomena might serve to attune hatching to increased surface water temperature which is associated with plankton production, the source of larval sustenance (Blaxter and Hempel 1966).

Any interference with oxygen transport could also reduce development. Thus, in conjunction with decreased incubation time of exposed eggs, shorter, less advanced larvae could be produced. That is, less time is available for larval growth or conversion of yolk into body tissue. However, possible differences in larval length resulting from decreased incubation time with greater exposure could be reduced or eliminated by increased metabolism resulting from higher air temperature. Since larval length is related to egg size, shorter larvae are possibly less viable than those from larger eggs, which have lesser food requirements, faster swimming speed, and a greater degree of thermal insulation (Marshall 1953). Those factors which tend to further reduce length compound difficulties encountered by emerging larvae.

Exposure also affects larval weight, which may be related to egg weight at spawning, and hence, to beach height. Because temperature and available oxygen also affect efficiency at which yolk is converted into body tissue, larval weight could be expected to be optimal at some degree of exposure, being decreased by either lower temperature with less exposure or greater hypoxia with more exposure. Although heavier larvae might be highest on the beach at spawning, this distribution could change by hatching, leaving heavier larvae at middle beach heights. Since eggs from all exposed beach levels may yield larvae of essentially the same length, heavier individuals possibly possess greater yolk reserves, and hence, may survive longer if their potential food source is late in appearing. The apparently large decrease in larval weight with greatest exposure coincides with a high prehatching mortality, and suggests a "critical point" in exposure is reached above which eggs stand little chance of normal development or survival. This upper limit seems to be near a 14-hr exposure per day or roughly the 3.5 m beach level above mean low tide.

From this study it seems that larger eggs from larger fish are better fitted to survive at higher levels of spawning. Reduction in mean spawner size by fishing such as noted by Cushing and Bridger (1966) could put smaller eggs further up the beach where they are less able to survive. Moreover, characteristics of emerging larvae might be so altered that viability is decreased or planktonic hazards increased. In consequence, a heavy fishing pressure on Pacific herring would imply a decreased average rate of survival via effects of intertidal exposure on spawn.

Acknowledgments

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Volatile Compounds Produced in Ground Muscle Tissue of Canary Rockfish (*Sebastes pinniger*) Stored on Ice[1]

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MILLER, A., III, R. A. SCANLAN, J. S. LEE, AND L. M. LIBBEY. 1972. Volatile compounds produced in ground muscle tissue of canary rockfish (*Sebastes pinniger*) stored on ice. J. Fish. Res. Bd. Canada 29: 1125–1129.

Volatile compounds associated with the progressive degradation of ground muscle tissue of canary rockfish (*Sebastes pinniger*) stored on ice were determined by combined gas–liquid chromatography and mass spectrometry. Compounds positively identified included dimethyl sulfide, n-propyl alcohol, acetaldehyde, propionaldehyde, acetone, ethyl alcohol, 2- and 3-pentanone, diacetyl, hexanal, 1-pentene-3-ol, 3-methyl-1-butanol, acetoin, trimethylamine, and dimethylamine. The following compounds were tentatively identified: propenal, octenal or octadienal, butyraldehyde, 3-methyl butanal, 2-butanone, and methyl vinyl ketone.

MILLER, A., III, R. A. SCANLAN, J. S. LEE, AND L. M. LIBBEY. 1972. Volatile compounds produced in ground muscle tissue of canary rockfish (*Sebastes pinniger*) stored on ice. J. Fish. Res. Bd. Canada 29: 1125–1129.

Nous avons déterminé par chromatographie en phase gaz-liquide et par spectrométrie de masse les composés volatiles associés à la dégradation progressive du tissu musculaire haché de *Sebastes pinniger* entreposé sur glace. Parmi les composés identifiés avec certitude, on compte les suivants: diméthyl sulfure, alcool n-propylique, acétaldéhyde, proprionaldéhyde, acétone, alcool éthylique, 2- et 3-pentanone, diacétyl, hexanal, 1-pentène-3-ol, 3-méthyl-1-butanol, acétoïne, triméthylamine et diméthylamine. Les composés suivants ont été identifiés provisoirement: propénal, octénal ou octadiénal, butyraldéhyde, 3-méthyl butanal, 2-butanone et méthylvinylcétone.

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ALTHOUGH numerous reports have dealt with the spoilage of rockfish during low temperature storage, much of the research was concerned primarily with organoleptic analyses and chemical indices of spoilage. Miyauchi and Stansby (1952) studied the rate of deterioration of several species of *Sebastes* during cold storage and observed that fillets from Pacific ocean perch (*S. alutus*) had the best keeping quality of all the Pacific coast rockfish examined. Canary rockfish (*S. pinniger*) equaled *S. alutus* in initial palatibility but deterioration was rapid and the fillets were inedible after 5 months. Liston et al. (1961) reported no significant difference among the spoilage rates of *S. alutus*, *S. melanops*, and *S. pinniger* during ice storage. The pattern of spoilage, indicated by organoleptic scores and chemical indices, was similar to that of gadoid fish. In the case of rockfish, total volatile acid and total volatile base determinations provided a useful measure of spoilage. Hillig et al. (1960a, 1961a) observed a high degree of correlation between organoleptic analyses and volatile acid number, formic and acetic acids, total volatile bases and trimethylamine (TMA) in iced ocean perch. Succinic acid and ethyl alcohol showed less correlation with organoleptic scores. Identical results were obtained for other groundfish; cod (Hillig et al. 1958), haddock (Hillig et al. 1959), flounder (Hillig et al. 1960b), and whiting and pollack (Hillig et al. 1961b).

Several investigations (Ota 1958; Mangan 1959a; Diemair and Schams 1962; Mendelsohn and Steinberg 1962; Groninger 1961; Wong et al. 1967) on the volatile constituents of various species of fish have been reported; however, only limited data are available for species of *Sebastes*. Therefore, this study was primarily initiated to identify the volatile compounds associated with the autolytic and progressive microbial degradation of raw rockfish — *S. pinniger* stored at 0 C.

[1]Technical Paper No. 3262, Oregon Agricultural Experiment Station.

Printed in Canada (J2441)

Materials and Methods

Sample Preparation

Noneviscerated canary rockfish (*S. pinniger*), obtained from coastal commercial sources, were packed in ice and stored at 0 C overnight. Fillets were removed and ground in a sterile meat grinder. The ground fish was placed in a glass jar in a shallow layer, covered with aluminum foil, capped and stored at 0 C (ice–water equilibrium). All samples were kept at ice temperature throughout the handling and processing operation. At 1, 7, 14, and 21 days, unless stated otherwise, the fish was mixed thoroughly and portions (5–10 g) were removed for subsequent chromatographic analysis.

Gas Chromatographs

Varian Aerograph (series 1200, 204, and 1400) and F & M Model 810 gas chromatographs were used throughout this investigation. The Varian Aerograph series 1400 instrument was equipped with an alkali flame ionization detector (AFID) which was routinely tuned for optimum performance as described by the manufacturer (Varian Aerograph, Walnut Creek, Calif.). The remaining instruments were equipped with flame ionization detector systems.

Gas Chromatographic Columns and Conditions

A stainless steel column (18 ft × ⅛ inch O.D.), containing 40–60 mesh Graphon (Cabot Corp., Billerica, Mass.) coated with 2% tetraethylenepentamine (TEP), was used in conjunction with the AFID for the selective separation and quantitative determination of dimethylamine (DMA) and trimethylamine (TMA). Ten milliliters of 10% NaOH were added to each 5-g portion of fish contained in screw-capped vials (Kimble No. 60057, size No. 1) and the amine contents were determined by equilibrium vapor analysis after heating at 60 C for 10 min (Miller et al. 1972).

An additional column (12 ft × ¼ inch O.D.), containing acid base washed Celite 545 (60–80 mesh) coated with 20% 1,2,3-tris(2-cyanoethoxy) propane (TCEP), was used with a gas entrainment, on-column trapping procedure (Morgan and Day 1965) for the identification of relatively low-boiling compounds. Seven milliliters of double, glass-distilled water were added to each 5-g portion of fish contained in Kimble screw-capped vials. When necessary, a few milligrams of 1-tetradecanol were added to control foaming. Each sample was tempered at 60 C for 5 min and the volatiles were condensed on the column packing using a nitrogen gas purge rate of 12 ml/min for 15 min. The sample size and nitrogen purge time were increased when more concentrated samples were required for mass spectral analysis.

The TCEP and Graphon plus TEP columns were operated isothermally at 60 C with a nitrogen gas flow rate of 30 ml/min. The detector and injector port temperatures were 210 and 190 C, respectively.

A capillary column (500 ft × 0.03 inch I.D.), coated with 8% Carbowax 20 M and 1% Versamid 900, was operated isothermally at 70 C for 5 min, then temperature programmed to 190 C at 1.15 C/min. The detector and injector port temperatures were 240 and 230 C, respectively.

Analysis of Volatiles Collected on Porapak Q

Volatile compounds from spoiling fish samples (10 g) were collected for subsequent capillary column, gas chromatographic-mass spectral analyses as follows: samples, contained in screw-capped vials, were purged with nitrogen (30 ml/min) at 60 C for 30 min and the volatiles were collected in acetone-washed, 100–120 mesh Porapak Q traps (102 × 6 mm I.D.) essentially as described by Tressl and Jennings (1972). The traps were heated at 50 C with a thermostatically controlled heat gun and water was removed under a stream of nitrogen (20 ml/min) for 60 min. The traps were reversed and the volatiles, transferred for 30 min at 140 C with nitrogen (20 ml/min) to an open tubular trap (150 × 1.25 mm I.D.) immersed in a slurry of dry ice in 2-methoxyethanol, were then introduced into the capillary column through a modified inlet system (Scanlan et al. 1968) while the column was connected to the high-vacuum system of the mass spectrometer (MS). Since relatively high-boiling compounds might be present, it was necessary to heat a section of the capillary tubing with electrical heating tape to prevent sample condensation (Scanlan et al. 1968). When TMA production was pronounced, all samples were acidified to *p*H 3–4 with HCl to convert the free amine to the nonvolatile acid salt before analysis with either the capillary or TCEP column.

The Porapak Q traps were conditioned at 190 C for 30 min and at 100 C for 24–48 hr with a nitrogen gas flow rate of 60 ml/min. Subsequent gas chromatographic analyses of conditioned traps revealed no significant quantitative or qualitative changes above the background response.

Mass Spectral Analysis

An F & M Model 810 gas chromatograph was used in conjunction with an Atlas CH-4 MS for all mass spectral analyses. The operating conditions for the MS were: electron potential, 70 ev; trap current, 12 μA; and accelerating voltage, 3Kv. Spectra were scanned from m/e 25 to m/e 250 in 2.5 sec. A micro-volume switching valve (Carle Instruments, Inc., Fullerton, Calif.) was installed between the MS and gas chromatograph to divert air from the ion-source during sample transfer.

Results and Discussion

Volatile compounds produced in *S. pinniger* stored at 0 C are listed in Table 1. Identifications were made by comparisons of mass spectra with reference spectra and, in most cases, relative retention times of authentic compounds, obtained by gas–liquid chromatography, were used to confirm the mass spectral identifications. Compounds positively identified included dimethyl sulfide, n-propyl alcohol, acetaldehyde, propionaldehyde, acetone,

ethyl alcohol, 2- and 3-pentanone, diacetyl, hexanal, 1-pentene-3-ol, 3-methyl-1-butanol, acetoin, TMA, and DMA. Octenal or octadienal was tentatively identified on the basis of mass spectral data. Additional evidence, based only on relative retention times, indicated the presence of butyraldehyde, 3-methyl butanal, 2-butanone, propenal, and methyl vinyl ketone. Several of the compounds listed in Table 1 have been reported in other groundfish, such as cod (Wong et al. 1967) and haddock (Mangan 1959b). In addition, Groninger (1961) reported the formation of acetoin in a species of *Sebastes* during refrigerated storage.

A typical chromatogram of volatiles from *S. pinniger* (5 g) stored at 0 C for 16 days is shown in Fig. 1. Compounds identified are listed as follows with respective peak numbers: (1) a silicone contaminant, (2) ethyl alcohol, (3) diacetyl, (4) n-propyl alcohol, (5) not identified, (6) hexanal, (7) 1-pentene-3-ol, (8) 3-methyl-1-butanol, and (9) acetoin. The mass spectrum for peak 5 (Fig. 1) indicated major ions at m/e 29, 43, 57, and 100; however, the relative intensities observed could not be correlated with known spectra. Although not shown in Fig. 1, 2- and 3-pentanone had retention times similar to that of diacetyl. Several additional compounds were detected but not identified because of inconclusive gas chromatographic and mass spectral data.

Although low molecular weight carbonyls, such as acetaldehyde and propionaldehyde, were formed

TABLE 1. Volatile compounds identified in *S. pinniger* during storage at 0 C.

Compound	Storage days	Method of identification[a]	Columns[b]
Dimethyl sulfide	7,14	RT–MS	I,II
n-propyl alcohol	10,14,21	RT–MS	III
Acetaldehyde	7,14	RT–MS	II
Propionaldehyde	7,14	RT–MS	II
Acetone	7,14,21	RT–MS	II,III
Ethyl alcohol	7,14,21	RT–MS	II,III
2-pentanone	14,21	RT–MS	II,III
3-pentanone	14,21	RT–MS	II,III
Diacetyl	10,14,21	RT–MS	II,III
Acetoin	10,14,21	RT–MS	II,III
Trimethylamine	21	RT–MS	I,III
Dimethylamine	15,20,30	RT–MS	I
3-methyl-1-butanol	10,14,21	RT–MS	III
1-pentene-3-ol	10,16,17	RT–MS	III
Hexanal	10,16,17	RT–MS	III
Octenal or octadienal	16,17	MS	III
Propenal	16,17	RT	II,III
Butyraldehyde	14,21	RT	II
3-methyl butanal	14,21	RT	II
2-butanone	14,21	RT	II
Methyl vinyl ketone	14,21	RT	II

[a]RT, retention time; MS, mass spectra.
[b]I, Graphon plus 2% TEP. II, 1, 2, 3-tris(2-cyanoethoxy)propane. III, Carbowax 20M and Versamid 900.

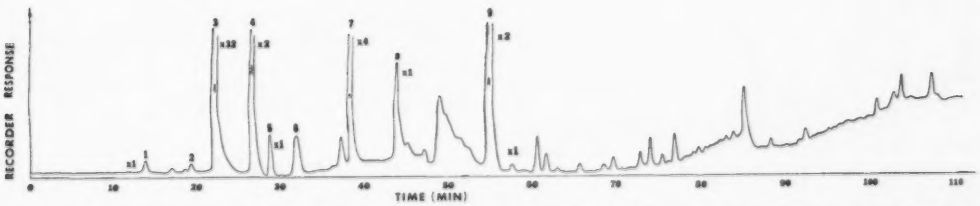

FIG. 1. Chromatogram of volatiles from *S. pinniger* stored at 0 C for 16 days. Column: 8% Carbowax 20M plus 1% Versamid 900 operated isothermally at 70 C for 5 min, then temperature programmed to 190 C at 1.15 C/min.

during the early stages of spoilage, the formation of relatively large amounts of higher molecular weight aldehydes, ketones, and alcohols occurred just before the samples were considered unacceptable. The quantity of several compounds appeared to increase with storage time to an apparent maximum, then decreased upon continued storage.

The development of TMA and DMA in ground flesh of *S. pinniger* stored at 0 C for 20 days is shown in Fig. 2. A slight increase in TMA content was observed between 0 and 6 days; however, subsequent TMA production was more pronounced because of the increased bacterial reduction of trimethylamine oxide. Earlier experiments, employing similar conditions, indicated that the natural flora apparently initiated a rapid phase of logarithmic growth at 6 days storage at 0 C (Lobben and Lee 1968). Therefore, the interval between 6 and 8 days appeared to be a critical period after which spoilage progressed rapidly. All samples were considered inedible after 9 days storage at 0 C. The DMA concentration remained relatively constant for 14 days but gradually increased to approximately 50 ppm at 20 days. DMA (70 ppm) was detected after 30 days storage at 0 C. Since DMA did not increase until the latter stages of spoilage, TMA appeared to be a more useful criterion of spoilage for minced muscle of *S. pinniger* stored at 0 C.

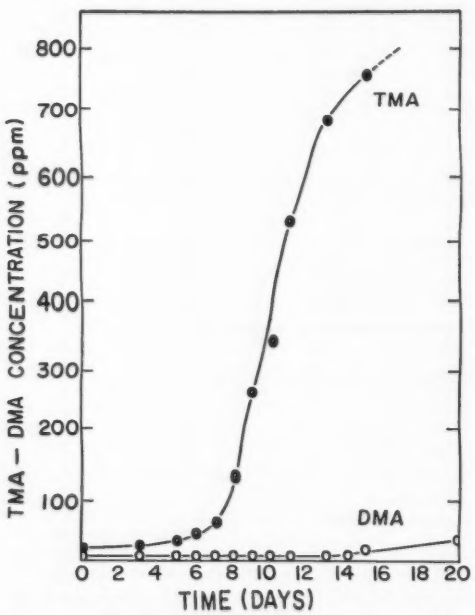

FIG. 2. Development of TMA and DMA in ground flesh of *S. pinniger* stored at 0 C.

Although an increase in TMA content can often be correlated with the decline in freshness of ocean teleosts, there are conflicting views regarding the reliability of TMA as a measure of quality or spoilage. Since TMA values are generally indicative of spoilage but are of less value in providing information on spoilage changes (Fieger and Friloux 1954), the TMA determination should be used in conjunction with other appropriate physical or chemical methods for the assessment of quality.

The usefulness of compounds such as acetoin, diacetyl, and 3-methyl-1-butanol as indicators of quality for canary rockfish was rather limited, since the increases in these particular compounds, as well as others, did not occur until the latter stages of spoilage. Although slight increases in low molecular weight carbonyls such as acetaldehyde, propionaldehyde, and acetone were observed during incipient deterioration, the use of these compounds as early indices of spoilage was also limited since they could not be easily correlated with quality changes. In fact, Diemar (1964) and Diemair and Schams (1962) suggested that carbonyls of low molecular weight are associated with fresh flavor.

Since lipid oxidation leads to the formation of a large number of volatile compounds, many of which have low threshold values and possess distinctive odors, a limited oxidation is often sufficient to cause flavor defects. The data presented for *S. pinniger*, substantiated by preliminary results obtained for *S. melanops*, indicated that products of lipid oxidation, such as hexanal, and, particularly, 1-pentene-3-ol, may be useful indicators of early spoilage. Although the latter components were detected within 6 days storage at 0 C, further investigation is needed to correlate quantitative changes with sensory evaluations.

The results presented herein indicated that TMA could be used as an index of spoilage for *S. pinniger*. Liston et al. (1961) and Hillig et al. (1958–61) also reported that total volatile base and TMA determinations provided a useful measure of spoilage for several species of groundfish, including rockfish. In addition, products of lipid oxidation, such as 1-pentene-3-ol, might be useful as early indicators of flavor defects. Since ground fish was used throughout this investigation, it should be noted that the rate and possibly the pattern of decomposition might vary in whole fish and fillets.

Acknowledgments

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Acidification of the La Cloche Mountain Lakes, Ontario, and Resulting Fish Mortalities[1]

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BEAMISH, R. J., AND H. H. HARVEY. 1972. Acidification of the La Cloche Mountain Lakes, Ontario, and resulting fish mortalities. J. Fish. Res. Bd. Canada 29: 1131–1143.

The loss of populations of lake trout (*Salvelinus namaycush*), lake herring (*Coregonus artedii*), white suckers (*Catostomus commersoni*), and other fishes in Lumsden Lake was attributed to increasing levels of acidity within the lake. An absence of fishes was also observed in nearby lakes. In some lakes, acid levels have increased more than one hundredfold in the last decade. The increases in acidity appear to result from acid fallout in rain and snow. The largest single source of this acid was considered to be the sulfur dioxide emitted by the metal smelters of Sudbury, Ont.

In 1971, *p*H measurements were taken from 150 lakes in the general study area 65 km southwest of Sudbury. Some 33 of these lakes showed a *p*H of less than 4.5 and were described as "critically acidic." An additional 37 lakes had a *p*H in the range of 4.5–5.5 and were termed "endangered" lakes.

BEAMISH, R. J., AND H. H. HARVEY. 1972. Acidification of the La Cloche Mountain Lakes, Ontario, and resulting fish mortalities. J. Fish. Res. Bd. Canada 29: 1131–1143.

On attribue à la hausse du niveau d'acidité la disparition des populations de touladis (*Salvelinus namaycush*), ciscos de lac (*Coregonus artedi*), catostomes noirs communs (*Catostomus commersoni*) et autres espèces du lac Lumsden. Les lacs avoisinants sont également dépourvus de poissons. Dans certains lacs, le niveau d'acidité a augmenté de plus de 100 fois au cours des 10 dernières années. Cette acidité accrue semble être le résultat de retombées acides des eaux de pluie et de neige. Les émanations de SO_2 des fonderies de Sudbury, Ont., sont considérées comme source principale de cet acide.

En 1971, nous avons effectué des mesures de *p*H dans 150 lacs du territoire sous étude, à 65 km au sud-ouest de Sudbury. Quelque 33 de ces lacs avaient un *p*H inférieur à 4.5 et ont été décrits comme ayant une "acidité critique". Trente-sept autres avaient un *p*H variant de 4.5 à 5.5 et ont été classés "lacs en danger".

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IN 1967 a study was initiated to examine a population of slow-growing white suckers (*Catostomus commersoni*) in Lumsden Lake, a small oligotrophic lake 65 km southwest of Sudbury, Ont., located within a series of quartzite ridges termed the La Cloche Mountains. By late 1968 it was determined that fishes in this lake and nearby lakes were rapidly disappearing. An examination of the causes of fish disappearance revealed that the *p*H of lakes in the area was surprisingly low and that the high acidity could not be explained by natural processes.

These remote lakes received no visible surface effluent of industrial origin and this raised the possibility of contamination from the atmosphere. It was known that fallout of airborne acid formed by the combination of sulfur dioxide and water vapor in the atmosphere could alter the *p*H of lakes and rivers (Gorham and Gordon 1960; Odén 1968). It was also known that large amounts of sulfur dioxide were emitted by the metal smelters in Sudbury, Ont. Therefore, in 1969 an examination of the *p*H of precipitation and monthly wind directions was undertaken to determine if the sulfur dioxide emitted in Sudbury could be the source of the acid that was entering Lumsden Lake and other lakes in the study area.

This report describes the acidification of lakes of the La Cloche Mountains and the loss of fish populations from Lumsden Lake. A subsequent report will discuss the effects of the increased acidity on the food supply and growth of the fishes in Lumsden

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Lake. Investigations to study the amount of sulfate and acid entering Lumsden Lake from precipitation are currently in progress.

Description of Lumsden Lake

The La Cloche Mountains of Ontario comprise a series of quartzite ridges or hills extending in an east–west direction, along the north shore of Georgian Bay. The southernmost ridge, Killarney Ridge, extends southwesterly in the direction of Manitoulin Island and within this ridge are located the Lumsden Lakes. Lumsden Lake is the lowermost in a chain of 6 lakes (known locally as Lumsden Lake and Lumsden II–VI Lakes, Fig. 1) located in Killarney Provincial Park at 46°01′N, and 81°26′W. None of the Lumsden Lakes is accessible by road and there is no habitation anywhere in this watershed.

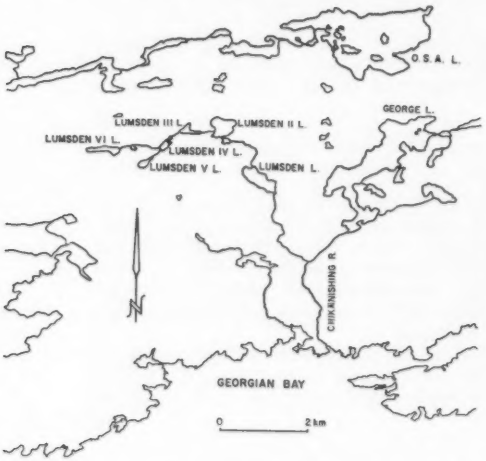

FIG. 1. Lakes and drainages of the study area, Killarney Ridge, southeastern La Cloche Mountains.

Lumsden Lake has an elevation of 241 m (m.s.l.), and was thus isolated as a lake about 6800 B.C. The lake has one major inlet with the water entering over a 3-m waterfall into a short, lake-level stream. The outlet contains a mass of log debris beyond which the stream wanders for 150 m before plunging down a 15-m waterfall. Due to these two falls it is not possible for fish to enter the lake through the outlet or leave the lake via the inlet stream. Below this, the outlet stream meanders for 2 km through a beaver meadow before connecting with the outlet of George Lake, the Chikanishing River.

Lumsden Lake is a moderately deep oligotrophic lake with a maximum depth (Zm) of 22 m, a mean depth ($\bar{Z}$) of 7 m, and a surface area of 20.9 ha. The water is clear, with little suspended matter, yielding a Secchi value of 8+m.

The dissolved and suspended solids, calcium, hardness, alkalinity, and all other anions and cations except sulfate were exceptionally low (Table 1).

Materials and Methods

Biological surveys of Lumsden Lake were first made by the Ontario Department of Lands and Forests in 1961 and 1963. Most of the detailed records associated with these surveys were lost in a fire in 1968. Commencing in 1966, the authors have continued annual fish surveys in this lake. An intensive biological study of the lake was begun in 1967, and in 1969 a program of monitoring lake acidity was initiated. Concurrently, similar studies were conducted on nearby George Lake, and during the years 1969–71 *p*H measurements were extended to some 150 lakes in the area.

Data are presented for catches of fish in Lumsden Lake by the Ontario Department of Lands and Forests. Such fish were caught in standard gangs of gillnets ranging in size from 15 to 100 mm stretched mesh. After 1963, gillnets used were of the survey type, 50 m long with 6 panels of 19, 25, 30, 33, 38, and 45 mm

TABLE 1. Water Chemistry of Lumsden Lake (all readings in *ppm* unless otherwise indicated).

	1968[a]	1970[a]		1968[a]	1970[a]
Colour (hazen units)	<5	–	Total phosphorus	0.04	0.005
Turbidity units	0.6	1.5	Soluble phosphorus	0.01	0.004
Total solids	32	40	Nitrate (as N)	0.055	0.14
Suspended solids	2	5	Nitrite (as N)	0.005	0.015
Dissolved solids	30	35	Sodium	0.7	0.6
Hardness as $CaCO_3$	16	10	Potassium	0.35	0.4
Alkalinity	5	4	Calcium	4	4
Bicarbonate alkalinity	5	4	Magnesium	2	0
Iron	0.1	0.05	Aluminium	0.44	0.17
Sulfite	–	2.0	Silica	0.12	–
Sulfate	12	11	BOD	0.8	–
Chloride	1	–			

[a]Analyses done by Ontario Water Resources Commission.

stretched mesh. In addition, 33-m commercial-type gillnets were used, ranging in mesh size from 19 to 127 mm. A series of trapnets of mesh sizes 0.8–38 mm square mesh (Beamish MS 1972) were operated in Lumsden Lake from 1967 to 1969. In 1968 and 1969, surveys were made of the two lakes up stream of Lumsden (Lumsden II, Lumsden III) and in 1970 netting was conducted in O.S.A. Lake.

The ages of white suckers in Lumsden Lake were determined from pectoral fin sections (Beamish MS 1970; Beamish and Harvey 1969). The number of white suckers in Lumsden Lake was estimated by the Petersen method — that is, the marking, release, and subsequent capture of marked and unmarked animals (details in Beamish MS 1970). The tag design and its suitability were described by White and Beamish (1972). In 1968, as part of an experiment to compare growth of white suckers in George and Lumsden Lakes, 107 tagged white suckers of George Lake origin were introduced into Lumsden Lake. An additional 64 tagged George Lake suckers were released into George Lake as controls. Suckers introduced into both lakes were "aged" using scales, and ranged from 1^+ to 3^+ years. In the spring of 1966 and 1967, H. H. Harvey introduced a total of 4000 pink salmon fry (*Oncorhynchus gorbuscha*) into Lumsden Lake. In 1966 the outlet stream was screened with 5-mm mesh wire.

Measurements of *p*H by the Ontario Department of Lands and Forests prior to 1968 were made with a Helige comparator. Acidity was measured in the field using a Sargent-Welch Model PBL portable *p*H meter, which was standardized at *p*H 7.0 and 4.0 before and after each series of readings. Up to 1970, field measurements were repeated on shore with a Fisher Model 310 expanded-scale *p*H meter. Measurements were made promptly in the field, as water samples from the La Cloche Lakes changed *p*H on standing (Beamish MS 1970); for this reason, *p*H measurements on stored samples were not quoted in this report.

Rainwater was collected in BOD bottles through a glass funnel covered with a fine-mesh nylon netting. Hydrogen ion concentration was measured immediately after rainfall. Measurements of snow *p*H were made by exposing a vertical section of the snow covering, then inserting 500-ml glass beakers into the snow section at specific depths. Samples were then heated slowly to melt the snow and the *p*H taken when enough water was present to immerse the electrodes. Several readings were taken from each sample using the two *p*H meters, and corrections were made for temperature.

Results

Fish Studies

Early settlers in the Killarney area fished Lumsden Lake for lake trout (*Salvelinus namaycush*), and this fishery continued until 1965. Prior to 1960, anglers also caught burbot (*Lota lota*) and reported yellow perch (*Perca flavescens*) from the stomachs of lake trout (Table 2). Survey fishing by the Ontario

TABLE 2. Fishes present, relative abundance, and fishing effort, Lumsden Lake 1961–71. Gillnet catches in parentheses. A, abundant; P, present; R, rare; NC, none caught; NR, no record.

	1961[a]	1963[a]	1966	1967	1968	1969	1970	1971
White sucker (*Catostomus commersoni*)	NR(0)	NR	A(179)	A(315)	R(3)	NC(1)[b]	NC(0)	NC(0)
Lake herring (*Coregonus artedii*)	A(62)	P	A(37)	A(1323)	P(212)	R(65)	NC(0)	NC(0)
Lake trout (*Salvelinus namaycush*)	P(7)	P	NC(0)	R(1)	NC(0)	NC(0)	NC(0)	NC(0)
Lake chub (*Hybopsis plumbeus*)	NR(0)	NR	A(−350)	A(−195)	A(−225)	A(−95)	(A−600)	P(4)
Trout-perch (*Percopsis omiscomaycus*)	NR(0)	NR	NC(0)	NC(0)	P(8)	R(1)	NC(0)	NC(0)
Slimy sculpin (*Cottus cognatus*)	NR(0)	NR	NC(0)	R(1)	NC(0)	NC(0)	NC(0)	NC(0)
Burbot[c] (*Lota lota*)	NR(0)	NR	NC(0)	NC(0)	NC(0)	NC(0)	NC(0)	NC(0)
Yellow perch[c] (*Perca flavescens*)	NR(0)	NR	NC(0)	NC(0)	NC(0)	NC(0)	NC(0)	NC(0)
Meters of gillnet <65 mm (stretched mesh) over 24-hr period	180	310	150	3000	1160	4275	460	600
Total meters of gillnet, all mesh sizes, over 24-hr period	460	500	430	8750	1250	4275	460	1065

[a]Sampling conducted by the Ontario Department of Lands and Forests. Catch records for 1963 destroyed by fire.
[b]Single fish caught was a tagged individual introduced from George Lake.
[c]Reported by anglers and local residents as present prior to 1960.

Department of Lands and Forests in 1961 and 1963 yielded lake trout and lake herring (*Coregonus artedii*).

Fish studies by the authors during the period 1966–71 were concentrated on the populations of the larger fishes, the white sucker, lake herring, and lake trout. The smallest net meshes also caught three small species, the lake chub (*Hybopsis plumbeus*), trout-perch (*Percopsis omiscomaycus*), and slimy sculpin (*Cottus cognatus*). Gillnet catches were used to indicate the abundance of all species of fishes (Table 2). Trapnets captured white suckers, lake chub, and trout-perch. Therefore, qualitative estimates of abundance (abundant, present, rare) for these three species were based on both gillnet and trapnet catches. In most cases lake chub and trout-perch were released unmarked back into the lake; consequently, it was not possible to determine the exact numbers of these two species captured in trapnets and qualitative estimates based on a total catch of gillnets and trapnets may be high. All white suckers released from trapnets were marked with a tag or a pelvic fin clip. In 1968, of 29 Lumsden Lake suckers captured, 26 were taken in trapnets. Thus, in relation to catches in 1966 and 1967, Lumsden suckers were termed rare in 1968 (Table 2).

White suckers in Lumsden Lake were slow growing, maturing at age 3 and a fork length of 110–130 mm, and none older than 7 years were captured there during this study. In 1967 the population of mature white suckers was estimated to be 1184 with 95% confidence limits of 889–1479, or approximately 1500 fish including immatures (Beamish MS 1970). Of these, some 344 were removed during 1967 and 1968, leaving at that time more than 800 mature or more than 1100 mature and immature white suckers in the lake; none of these were captured after October 5, 1968.

Lumsden suckers were observed spawning in the lake on June 3, 1966. In 1967, daily trapnet catches from May 24 to June 25 did not include any female suckers in spawning condition. Also, no spawning was observed in the lake or in inlet or outlet streams during this period. Tagged and untagged females which might have been ready to spawn in May were found to be resorbing their ova in June. No young-of-the-year suckers were captured in 1967 and no one-year-old suckers were caught in 1968. Also, in 1968 no suckers were captured in spawning condition. The absence of 5-year-old suckers in 1967, the 1962 year-class, indicated that reproduction had not been successful in the past.

Extensive fishing in Lumsden Lake, 1966–71, failed to capture any of the 4000 pink salmon introduced in 1966 and 1967. Of the 107 George Lake suckers introduced into Lumsden Lake in 1968, 23 were recovered in gillnets or trapnets in June–July and removed from the lake. In addition, 12 were captured in trapnets and returned to Lumsden Lake in good condition. Only one of the possible remaining 84 introduced suckers was recaptured in the extensive netting program of 1969 (Table 2) and none in 1970–71. Five of the 64 control fish that were released into George Lake were recaptured in 1969, two in 1970, and one in 1971 with less fishing effort than had been applied to Lumsden Lake. The surveys of 1961 and 1963 by the Ontario Department of Lands and Forests confirmed the presence of lake trout in Lumsden Lake. The netting of 1967 captured a single individual, but no lake trout were captured in the lake after 1967. Some 5750 m of large-mesh gillnet suitable for the capture of lake trout and burbot were set over a period of 3 weeks and failed to yield a single fish. In the fall of 1967, catches of white suckers in Lumsden Lake declined drastically. Despite an increase in fishing effort, catches of lake herring declined in 1968 and again in 1969; and none of this species was caught in 1970–71. During the period 1967–69, no immature lake herring were captured, in contrast to similar netting efforts in George Lake that yielded both immature and mature lake herring.

Fishing effort was not directed specifically at trout-perch and slimy sculpin. However, on the basis of the very small numbers caught in fine-mesh gillnets, it appeared that these species were not abundant in 1966–68, and were rare or absent in 1969–71 (Table 2). The lake chub was an abundant species in 1966–69, and was the only species found in Lumsden Lake after 1969. Burbot and yellow perch were reported by local anglers as being present in the lake prior to 1961, but none were captured in gillnets or trapnets during the study.

In the extensive netting survey of the lakes upstream from Lumsden in 1968–69, the total catch in Lumsden II was one white sucker, six lake herring, and 25 lake chub. No fish were caught in Lumsden III in 1969. In 1970, 500 m of gillnet failed to capture any fish in nearby O.S.A. Lake. Up to the mid-1960 s, local fishermen had conducted a successful winter sport fishery for lake trout in Lumsden, Lumsden II, and O.S.A. Lakes.

Lake Acidification

Lumsden Lake decreased in *p*H from 6.8 in September 1961 to 4.4 in August 1971 (Table 3); this represented an increase of more than one hundredfold in the amount of hydrogen ion in the lake over 10 years. This high level of acidity was not unique to Lumsden Lake as indicated by the results of the 1971 measurements of the lakes up-

stream: Lumsden II, 4.0; III, 4.0; IV, 4.1; V, 4.0; VI, 4.0. Early measurements are not available for Lumsden Lakes II–VI, but for lakes II and III the decline of 0.6 *p*H units over 2 years as an annual change was similar to the annual change in Lumsden Lake (Table 3). Little vertical stratification of *p*H was observed in Lumsden and George Lakes; from 1969–71 some seasonal variation occurred in *p*H,

TABLE 3. Earliest and 1971 *p*H measurements on lakes in and to the east of the La Cloche Mountains.

Lake	Township	Date	*p*H	Avg. annual change in *p*H units
Broker[a]	Attlee	Sept./61[b]	6.8	−0.21
		Aug./71	4.7	
Carlyle	Carlyle	May/68[b]	5.5	−0.13
		Aug./71	5.1	
David	Stalin & Goschen	Aug./61[b]	5.2	−0.09
		Aug./71	4.3	
Freeland	Killarney	June/69	5.2	−0.20
		Sept./71	4.8	
George	Killarney	Sept./61[b]	6.5	−0.18
		Sept./71	4.7	
Grey[a]	Sale	Sept./59[b]	5.6	−0.13
		Sept./71	4.1	
Johnnie	Goschen & Carlyle	Aug./61[b]	6.8	−0.20
		Aug./71	4.8	
Kakakise	Killarney	June/68[b]	6.0	−0.10
		Aug./71	5.7	
Killarney	Killarney	Aug./69	4.5	−0.05
		Sept./71	4.4	
L & F 24	Carlyle	Sept./67[b]	6.0	−0.25
		Aug./71	5.0	
Lumsden	Killarney	Sept./61[b]	6.8	−0.24
		Aug./71	4.4	
Lumsden II	Killarney	June/69	4.6	−0.30
		Oct./71	4.0	
Lumsden III	Killarney	June/69	4.6	−0.30
		Oct./71	4.0	
Mahzenazing[a]	Carlyle & Humboldt	Sept./61[b]	6.8	−0.15
		Aug./71	5.3	
Nellie	Roosevelt	Sept./69	4.5	−0.05
		Aug./71	4.4	
Norway	Killarney	Sept./69[b]	4.5	0.00
		Aug./71	4.5	
O.S.A.	Killarney	/61[b]	5.5	−0.12
		Sept./71	4.3	
Spoon[a]	Kilpatrick & Humboldt	Sept./61[b]	6.8	−0.12
		Aug./71	5.6	
Sunfish[a]	Humboldt	Sept./61[b]	6.8	−0.24
		April/71	4.4	
Threenarrows	Killarney, Roosevelt, & Stalin	Nov./69[b]	5.2	0.00
		Aug./71	5.2	
Tyson[a]	Sale & Humboldt	Aug./55[b]	7.4	−0.16
		Aug./71	4.9	
Unnamed Lake[a] (46°01′30″N 81°24′W)	Killarney	June/69	5.7	−0.25
		Oct./71	5.2	
Mean of 22 lakes				−0.16

[a]Located east of the La Cloche Mountains.
[b]*p*H determined by the Ontario Department of Lands and Forests.

but the overall trend was toward lower *p*H values (Fig. 2).

Of the lakes in and to the east of the La Cloche Mountains, *p*H measurements were available from 11 lakes for 1961 or earlier (Table 3). Compared with *p*H values from 1971, all of these lakes showed a tenfold to more than a one hundredfold increase in hydrogen ion. Data was available subsequent to 1967 for an additional 11 lakes; two of these were unchanged and the remainder declined in *p*H (Table 3). Lakes that showed little change in *p*H (Table 3) were acidic when the initial sample was taken. Lakes within the La Cloche Mountains in 1971 had an average pH of 4.6 and were acidifying at an average annual rate of 0.15 *p*H units compared to 4.9 and a rate of 0.18 for the lakes immediately to the east. For all of the 22 lakes, the average *p*H in 1971 was 4.7 with an average annual decline of 0.16 *p*H units (Table 3).

For the lakes lying north of the La Cloche Mountains, *p*H readings prior to 1971 were available for 26 lakes (Table 4). Measurements of *p*H of seven lakes, spanning 10 years, showed virtually no change in three and a decrease of approximately one *p*H unit in four. For 19 lakes, *p*H measurements existed subsequent to 1966 and, compared with *p*H determinations in 1971, eight decreased in *p*H by more than 0.5, seven decreased less than 0.5, one was unchanged, and three increased (Table 4). In general, lakes listed in Table 4 had an average *p*H of 6.3 in 1971, and were less acidic and acidifying at a lesser rate than those within the La Cloche Mountains (Table 3).

The survey of 150 lakes in 1971 revealed that all lakes in the Lumsden chain were strongly acidic, ranging from *p*H 4.0 to 4.4 (Fig. 3). Of the 50-odd lakes tested within the eastern La Cloche Mountains, five had a *p*H less than 4.0, 20 ranged between 4.0 and 4.5, nine were between 4.6 and 5.0, and 13 were between 5.1 and 6.0. The remaining three lakes had *p*H values between 6.1 and 6.3. The majority of the lakes examined to the immediate north and east of the eastern La Cloche Mountains ranged between *p*H 4.1 and 6.0. The few lakes tested from the western La Cloche Mountains were less acidic, ranging from *p*H 5.4 to 7.2. Some 15 lakes spaced between the eastern edge of the La Cloche Mountains and Sudbury were acidic, having *p*H values of 3.8–5.6. Except for these 15 lakes, most lakes examined north of the general La Cloche region ranged from *p*H 6.1 to 7.8 and did not show the severe acidic conditions characteristic of the eastern La Cloche lakes.

The measurements of the *p*H of rainwater ranged from 3.6 to 5.5 (Table 5). Snow samples showed little vertical stratification, in general were uniformly

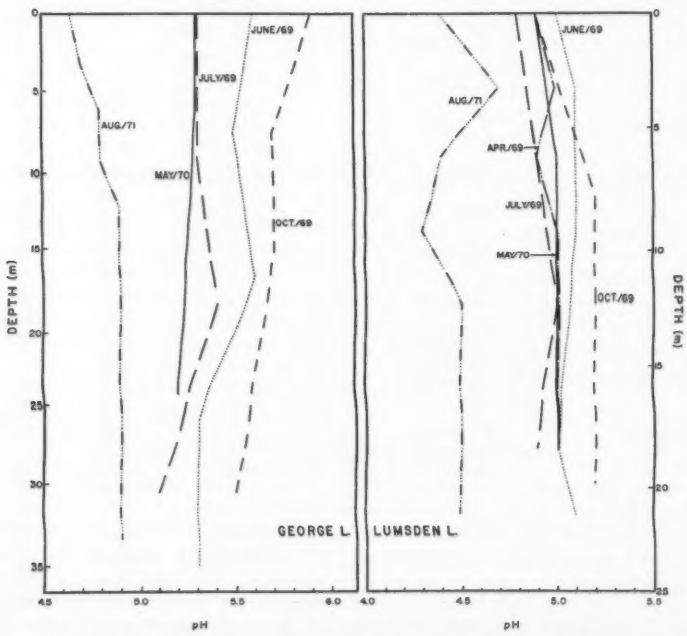

FIG. 2. *p*H profiles of Lumsden and George lakes, 1967–71.

TABLE 4. Earliest and 1971 *p*H measurements on lakes north of the La Cloche Mountains.

Lake	Township	Date	*p*H	Avg. annual change in *p*H units
Anderson	Merritt	Aug./60[a]	7.4	−0.09
		Oct./71	6.4	
Annie	Bevin & Sale	/61[a]	5.6	−0.09
		Aug./71	4.7	
Bear	Roosevelt & Dieppe	Aug./68[a]	6.5	−0.07
		Aug./71	6.3	
Brazil	Foster	Aug./67[a]	7.5	−0.20
		Aug./71	6.7	
Deerhound	Curtin	Sept./68[a]	7.0	−0.10
		Aug./71	6.7	
Elizabeth	Foster	Sept./68[a]	6.5	+0.33
		Sept./71	7.5	
Frank	Goschen	/60[a]	6.9	−0.03
		Oct./71	5.6	
Fox	Goschen	July/60[a]	6.1	−0.07
		Sept./71	5.3	
Griffin	Merritt	Aug./60[a]	7.8	−0.10
		Oct./71	6.7	
Hannah	Foster, Truman, Curtin, & Roosevelt	Aug./68[a]	7.0	−0.10
		Aug./71	6.7	
Hanwood	Roosevelt	Aug./67[a]	7.0	−0.25
		Oct./71	6.0	
Lang	Curtin	Aug./68[a]	6.5	+0.10
		Oct./71	6.8	
Leech	Roosevelt	Aug./67[a]	6.5	−0.13
		Oct./71	6.0	
Little Bear	Roosevelt	Aug./68[a]	6.5	−0.27
		Oct./71	5.7	
Little Hannah	Truman	Aug./68[a]	7.5	−0.33
		Aug./71	6.5	
Little Panache	Louise & Dieppe	July/68[a]	8.5	−0.35
		May/70	7.8	
Long	Eden, Waters, & Broder	Nov./69[a]	6.5	+0.15
		Sept./71	6.8	
Loon	Merritt & Foster	Sept./68[a]	6.5	0.00
		Oct./71	6.5	
Plunge	Roosevelt	Aug./68[a]	6.6	−0.20
		Oct./71	6.0	
St. Leonard	Foster	Sept./68[a]	6.8	−0.03
		Aug./71	6.7	
Simon	Graham	Aug./60[a]	6.1	+0.03
		Sept./71	6.4	
Spring	Merritt	Aug./66[a]	7.0	−0.16
		Oct./71	6.2	
Stratton	Foster	Sept./68[a]	7.0	−0.10
		Aug./71	6.7	
Walker	Truman & Roosevelt	Aug./68[a]	6.5	−0.07
		Aug./71	6.3	
Whitefish	Whitefish Indian Reserve	Aug./60[a]	6.3	+0.01
		Oct./71	6.4	
White Oak	Tilton & Halifax	Nov./69[a]	4.2	−0.05
		Oct./71	4.1	
Mean of 26 lakes				−0.08

[a] *p*H determined by the Ontario Department of Lands and Forests.

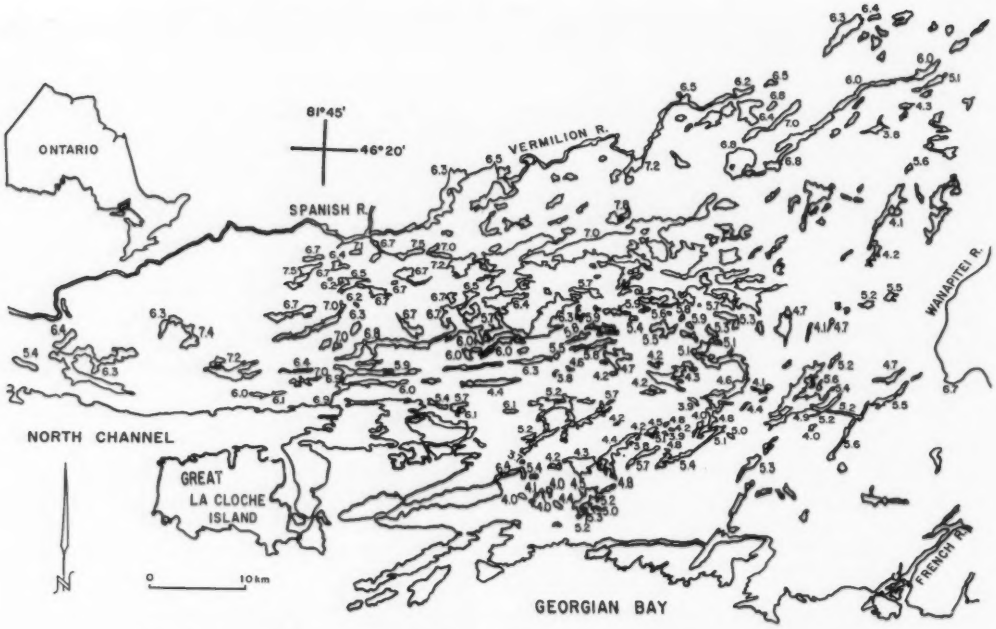

FIG. 3. *p*H values recorded in 1971 from lakes in the La Cloche Mountain region.

acidic, and, in the Lumsden and George Lake areas, were strongly acidic, ranging from *p*H 2.9 to 3.8.

Wind direction in the Sudbury area was recorded by the Department of Transport (monthly record meteorological observations in Canada) as the number of hours per month that the wind blew from each of 16 points of the compass. From these monthly records the percentage of wind-hours from each of the 16 compass directions was calculated for the decade 1961–70 (Table 6). Lumsden Lake lies in the direction S to SW of Sudbury, and thus in the path of Sudbury winds from compass points N, NNE, and NE. These three compass points account for an appreciable part of the total hours of recorded wind direction. For the decade 1961–70, approximately 24% of winds (as wind-hours) at Sudbury were from the N, NNE, and NE directions.

Discussion

Population Changes

A drastic decline in the abundance of fishes in Lumsden Lake took place over the period 1961–71. The decline in numbers of the various species occurred at different times during the study (Table 2), indicating that all species were not equally affected by similar levels of what was stressing the populations. Burbot disappeared prior to 1966, followed by the disappearance of lake trout in 1967, slimy sculpin in 1967–68, white suckers in 1968–69, lake herring and trout-perch in 1969–70. The lake chub appeared to be the last fish species remaining in the lake, and its numbers were declining in 1971. If yellow perch existed in the lake, they also disappeared before the commencement of this study in 1966. The majority of the George Lake white suckers introduced into Lumsden Lake in 1968 appeared to survive for only a few months; only one of these was captured 1 year after its introduction. The relative success in capturing the control suckers in George Lake from 1969–71 indicated that handling and marking probably were not major reasons for the lack of survival.

Loss of the white sucker population was preceded by failures of the species to reproduce. No immature lake herring were captured, indicative that reproduction in this species also might not have been successful in the years immediately preceding their disappearance. In 1967 the failure of white suckers to reproduce appeared to result from the inability of females to attain spawning condition.

The disappearance of fish from Lumsden Lake was not unique. No fish were found in Lumsden III Lake, very few fishes were present in Lumsden II

Lake, and limited netting yielded no fish in O.S.A. Lake. These results were interpreted as evidence that the factor or factors adversely affecting the fishes of Lumsden Lake were probably widespread.

TABLE 5. *p*H of rainwater and snow in the vicinity of George and Lumsden lakes, 1969–71.

Location	Date		*p*H
	Rainwater		
Island near main inlet of George Lake	May 17/69		4.0
	May 19/69		5.5
	June 1/69		4.7
Beach near outlet of George Lake	May 15/70		4.0
	Oct. 9/71		3.6
	Snow		
George Lake			
Open field, south shore	Feb. 18/70	surface	2.9
		10 cm	3.0
		20 cm	3.4
		40 cm	3.3
	March 13/70	surface	3.7
		30 cm	3.8
		50 cm	3.8
Birch stand, south shore	Feb. 18/70	surface	3.4
		15 cm	3.4
		30 cm	3.35
		45 cm	3.5
Lumsden Lake			
Birch stand, east shore	Feb. 18/70	surface	3.3
		12 cm	3.3
		24 cm	3.0
		36 cm	3.0
	March 14/70	35 cm	3.8
		45 cm	3.8

It is important to note that both sport and nonsport fishes have disappeared from the lakes. Thus, while it is possible to argue that anglers were responsible for the decline in numbers of lake trout, it is very doubtful that anglers can be considered responsible for the disappearance of white suckers, lake herring, trout-perch, and slimy sculpin.

In the period 1961–71, the *p*H of Lumsden Lake declined from 6.8 to 4.4 and it is suggested that this increase in acidity was responsible for the stress exerted on the fishes, which ultimately resulted in their disappearance from the lake.

ACID TOLERANCE

The effects of high concentrations of hydrogen ion on the physiology of fishes has not been adequately studied. There is, however, sufficient information available to allow some general conclusions to be made concerning the range of acidity that is acceptable for fishes. After reviewing the literature, the EIFAC Working Party on Water Quality (1969) concluded a *p*H range of 5–9 was not directly lethal for fishes. Doudoroff and Katz (1950) concluded that most freshwater fishes could live in waters above *p*H 5.0, but questioned the suitability of waters close to 5.0 for successful reproduction of the more sensitive species. The National Technical Advisory Committee (1968) set the minimum desirable *p*H at 6.0 for aquatic organisms. McKee and Wolf (1963) stated that the optimum *p*H of water for fish culture was in the range of 6.7–8.6, with a practical extreme lower limit of 6.3. They reviewed some of the literature on acid tolerance and recorded fish surviving in water of *p*H 3.3; however, tolerable lower limits for most fish were close to 5.0. Lloyd

TABLE 6. Annual percentage of wind-hours as recorded at Sudbury, Ont.

Direction	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970
N	10.37	9.12	10.38	9.70	8.51	9.85	9.70	9.89	10.57	8.64
NNE	8.00	11.16	6.74	5.46	7.16	6.63	7.85	6.85	9.33	8.06
NE	7.86	8.48	6.97	5.54	6.43	5.81	5.22	6.09	7.37	7.07
ENE	2.30	2.91	2.50	1.94	2.88	3.57	3.36	3.27	3.49	3.42
E	3.08	3.60	2.33	3.91	4.70	2.67	4.67	3.65	2.74	4.23
ESE	2.35	3.09	1.37	4.12	3.18	2.31	3.22	3.28	2.03	3.54
SE	5.42	4.24	3.05	5.80	5.06	3.83	4.92	4.65	3.19	4.79
SSE	4.47	5.17	4.13	4.54	4.83	4.18	5.45	5.41	4.15	6.74
S	8.67	7.28	7.67	9.08	9.62	9.02	9.92	9.05	8.77	9.05
SSW	5.85	5.77	6.51	6.91	7.76	5.53	9.04	7.81	7.35	7.08
SW	10.36	9.85	12.40	10.12	9.05	8.65	8.59	7.68	7.98	7.03
WSW	5.97	5.37	7.48	7.41	6.21	7.58	7.13	7.55	7.38	6.73
W	6.23	5.13	7.19	7.08	6.93	8.90	6.65	7.78	8.29	6.53
WNW	4.75	5.21	5.52	4.71	5.97	6.78	4.47	5.69	5.25	4.83
NW	8.18	6.97	9.38	7.91	5.75	7.60	4.81	5.09	5.59	5.31
NNW	6.15	6.64	6.36	5.76	5.95	7.08	5.00	6.27	6.52	6.95

and Jordan (1964) found 50% of rainbow trout (*Salmo gairdneri*) died at *p*H 4.5 in 4–10 days, depending on the free carbon dioxide content of the water. Swingle (1961) reported waters ranging in *p*H from 5.4 to 3.6 were toxic to various species of pond fishes, and that near-lethal levels of acidity could affect reproduction. In water acidified to *p*H 4.0 with sulfuric acid, 50% of white suckers died in 12 days (Beamish 1972). The acid danger point for common carp (*Cyprinus carpio*) is reported from 4.8 (Schäperclaus 1933) to 5.4 (California Water Pollution Control Board 1952). Fish mortalities were observed when an acid discharge into the River Mörrumsen decreased the *p*H from 6.0 to 4.0–4.5, but ceased when the effluent was treated to bring the *p*H up to 5.0 (Vallin 1962). Bream (*Abramis brama*), roach (*Rutilus rutilus*), and perch did not breed in Lake Sysmäjärvi at *p*H 4.2–4.4 (Ryhänen 1961). Hultberg and Stenson (1970) rotenoned two lakes in southwest Sweden that appeared to have been acidified by atmospheric fallout of sulfuric acid. The lake, with a *p*H of 4.6, contained a total of 26 pike (*Esox lucius*), all of older age-classes, and 19 eels (*Anguilla anguilla*). Yellow perch, known to have existed in the past, were no longer present. The second lake, *p*H 4.8, contained only seven eels and one perch. The authors attributed the absence of young pike, the loss of perch, and the reduced numbers of the remaining species to the high acid content in the lakes.

There were no reliable *p*H determinations for Lumsden Lake from 1962 to 1968. However, on the basis of the information available in the literature it was concluded that the change in *p*H from 6.8 in 1961 to 4.4 in 1969 and 1971 represented an increase in the concentration of acid that was responsible for the decline in fish abundance. Similarly, the acid levels recorded in Lumsden II, III, and O.S.A. lakes appear to be sufficiently high to cause fish mortalities. In March, 1969, George Lake had a *p*H of 5.0 compared to 4.4 in Lumsden Lake. The increased acidity in Lumsden Lake appeared to be the reason the George Lake suckers introduced into Lumsden Lake disappeared, while the control suckers reintroduced into George Lake survived. The observations that increasing levels of acid in Lumsden Lake affected the reproduction of fishes prior to their disappearance and that all species were not equally resistant to similar levels of acidity are consistent with the observations of other workers. It is feasible that the resistance to acid is decreased by the interaction of acid with other compounds in the lake. High levels of heavy metals have been detected in the precipitation in the study area and vicinity (R. J. Beamish unpublished data; Gorham and Gordon 1963; J. R. Kramer personal communication). However, at present there is not sufficient reliable information to make a meaningful statement about the possible interaction of acid and other toxic substances.

Source of Acid

Natural production of acid could result from oxidation of pyrite or through breakdown of plant material. Neither of these processes appeared to be determining the *p*H of Lumsden Lake. Iron content of the water of this lake was extremely low (0.05–0.1 ppm), for example, in relation to sulfate (11–12 ppm). Sources of organic acids, such as bogs, were not common in the Lumsden chain, and the waters of beaver ponds in the vicinity of George and Lumsden Lakes in most instances were higher in *p*H than the lakes into which they drained. Clearly, it would be difficult to explain the recent and simultaneous acidification of these lakes on the basis of natural changes. A profound increase in acidity has occurred in these lakes in recent years (Table 3). It is unreasonable to conclude that these lakes have maintained *p*H levels suitable for fishes for 2000–9000 years and that natural phenomena have produced large and widespread increases in lake acidity only in the past decade.

Unnatural acidification of lake water could result from acid fallout in rain and snow, such as through conversion of sulfur dioxide to acid. It is becoming evident that atmospheric precipitation can exert an important or even a controlling influence on the chemistry of some lakes (Junge 1958; Gorham 1961; Tejning MS 1967; Schindler 1971). Several researchers have demonstrated the importance of rain and snow as importers of ions to lakes: Eriksson (1955) for Swedish lakes, Gorham (1958) in Britain, Gorham and Gordon (1958) in the area of Sudbury, Ont., and Gorham (1961) for Finnish waters. In the English Lake District, Gorham (1958) found small lakes on volcanic rock with very low dissolved salt content, and he concluded that half of the calcium, four-fifths of magnesium, and almost all of the chloride, sulfate, sodium, and potassium were contributed by rain. Acid precipitation entering Sweden was attributed by Barrett and Brodin (1955) to combustion acids, primarily sulfuric, produced in the densely populated and industrial regions of Central Europe. That such precipitation has resulted in the acidification of Scandinavian freshwaters has been documented by Odén and Ahl (1970). Snow was acidic (*p*H 4) and they claimed the resulting snow melt caused acid pulses in many rivers. The authors of the Swedish report (Anon. 1971) estimated that, if present rates of acidification continue, about one-half of Sweden's rivers will be in a *p*H range of 5.5–5.0 in less than 50 years, and they consider this range to be biologically critical.

It is known that sulfur dioxide can be transported in gaseous phase far from its industrial point of origin (Junge and Ryan 1958). The average life of a sulfur dioxide molecule in the atmosphere was estimated by Meetham (1950) to be 9–10 hr. Recently, Rodhe (1972) suggested that in Northern Europe anthropogenic sources of sulfur can remain in the atmosphere between 2 and 4 days. In the Swedish report (Anon. 1971), the authors claim sulfur fallout can occur up to several thousand kilometers from the emission source. Also, it has been demonstrated that sulfur dioxide can form sulfuric acid in the atmosphere (Thomas 1962; Waller 1963; Commins 1963; Foster 1969). Thus it is clear from the literature that sulfur dioxide can be considered to be a source of acid in precipitation and that the fallout of this acid can occur over great distances.

Mining in the Sudbury area began in earnest in 1886, with two furnaces in operation at Copper Cliff and two furnaces under construction at other mine sites. In 1916 an estimated 600,000 tons of sulfur dioxide was being released annually into the atmosphere (Anon. 1917). By 1940–45 the amount of sulfur dioxide released had reached 2.76 million tons annually (Anon. 1947). Since then, both production and sulfur recovery have increased. Based on the figure of 5200 tons of sulfur dioxide released daily by the smelter at Copper Cliff (Minister's Order, The Hon. G. A. Kerr, July 1970), 1000 tons per day at Coniston, and an estimated 1000 tons per day at Falconbridge (an operation the size of Coniston), it was estimated that the amount of sulfur dioxide released into the atmosphere in 1969 was 2.6 million tons.

Ida et al. (1966) have shown an inverse relation existed between the amount of sulfur dioxide fallout and wind speed. Hence, on windy days less sulfur dioxide fell near the source of emission and presumably a greater proportion was carried for greater distances. From 1961–70, approximately 24% of the winds (as wind-hours) from Sudbury blew in the direction of the study area. Snow was acidic and some 39% of the winds into the area occurred in the winter months December–March. Thus it was possible for the large amounts of sulfur dioxide released in Sudbury to be carried into the area of the La Cloche Mountains.

The effects of sulfur dioxide fallout have been studied on both vegetation and waters in the immediate vicinity of Sudbury. The damage of smelter fumes on terrestrial plants has been documented thoroughly (Linzon 1958; Gorham and Gordon 1960; Dreisinger and McGovern 1969). Gorham and Gordon (1960) tested 102 ponds and lakes and noted sulfate concentrations were high within a 5-mile radius of the smelters and at that time were considered negligible beyond 15 miles (24 km). Many of the polluted waters were strongly acidic, with *p*H values as low as 3.3. It appears that concentrations of acid increased in the study area during 1960–71 to the extent that damage to lakes is now apparent, whereas prior to 1960 it was observed only in waters in the vicinity of the Sudbury smelters.

Lake Changes

Lakes in the eastern La Cloche Mountains were extremely acidic. While Table 3 does not represent a random sample of lakes, all *p*H readings that were available prior to 1970 have been listed. Without exception, all lakes tested before 1969 showed a decrease in *p*H by 1971. The average annual decrease in *p*H of these lakes (Table 3) is greater than the average rates observed by Odén and Ahl (1970) for waters in Sweden. Lakes in the eastern La Cloche Mountains have predominantly quartzite drainages resulting in low alkalinities. The very low buffering capacity (Lumsden Lake alkalinity, 4–5 ppm) reduces their ability to neutralize acid influxes, especially acid snow melt. It is noteworthy that as the buffer in such a lake is exhausted, the further addition of equal amounts of acid will result in progressively more rapid acidification. Conversely, with exhaustion of the principal buffers, acidification may continue even though the acid intake of the lakes be reduced.

It is known that carbonate-bearing rocks outcrop in the region north of the western and central La Cloche Mountains. This potential source of buffer may explain, in part, why less acidic *p*H readings are found in this area (Fig. 3) and lakes are acidifying at a slower rate (Table 4). Because many factors can modify the effects of acid fallout, further comments concerning the distributions of *p*H readings in Fig. 3 must be reserved until more lakes are tested and more information is collected on the chemistry and geology of lakes in the La Cloche Mountains and surrounding areas.

The *p*H determinations of precipitation in the study area indicated some "acid" rains occurred and that snow was strongly acidic (*p*H 3.8). Thus, airborne sulfur dioxide entering the lakes as acid precipitation and runoff is regarded as the immediate cause of acidification of the study lakes and the resulting loss of fishes. Of the 150 lakes illustrated (Fig. 3), some 33 showed a *p*H of less than 4.5, and on the basis of the effects of such acidities on aquatic organisms such as fishes, these lakes are described as "critically acidic". An additional 37 lakes had *p*H values in the range of 4.5–5.5, and these lakes should be considered as "endangered lakes".

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Swimbladder Morphology and Specific Gravity of Myctophids off Oregon

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Three general types of swimbladders were found in the eight species of myctophids studied: gas-filled, fat-invested, and atrophied or reduced. Small specimens of all species had thin-walled, gas-filled swimbladders. Large specimens of *Stenobrachius leucopsarus* had fat-invested swimbladders and large *Diaphus theta* had either gas-filled or atrophied swimbladders, as found by other workers. Large *Tarletonbeania crenularis* had either gas-filled or reduced swimbladders, large *Lampanyctus ritteri* and *L. regalis* had reduced swimbladders, and large *Stenobrachius nannochir* had fat-invested swimbladders. *Protomyctophum thompsoni* and *P. crockeri* retained gas-filled swimbladders.

High body lipid content was found in *S. leucopsarus*, *S. nannochir*, *L. ritteri* and *D. theta*, and low lipid content was found in the other four species. Myctophids with high lipid content had specific gravities close to that of sea water (1.026–1.030). *Tarletonbeania crenularis* with a reduced swimbladder had a specific gravity of 1.088. *Lampanyctus regalis* had a lower specific gravity (1.040) due to high water content of the tissue.

The swimbladder to body volumes in *S. leucopsarus* and *D. theta* were inversely related to body size and lipid content, indicating that lipids assume the primary buoyancy function as the gas-filled swimbladder regresses with age. This change may eliminate the physiological constraints imposed by a gas-filled swimbladder and permit the more extensive diel vertical migrations of adults.

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Nous avons identifié trois types généraux de vessie gazeuse chez huit espèces de Myctophidae: remplie de gaz, revêtue de graisse et atrophiée ou réduite. Les petits individus de toutes les espèces ont une vessie à paroi mince, remplie de gaz. Les grands spécimens de *Stenobrachius leucopsarus* ont une vessie gazeuse recouverte de graisse, et les grands *Diaphus theta* ont une vessie soit remplie de gaz, soit atrophiée, comme l'ont remarqué d'autres chercheurs. Les *Tarletonbeania crenularis* de grande taille ont une vessie remplie de gaz ou réduite, les grands *Lampanyctus ritteri* et *L. regalis* ont une vessie gazeuse réduite, alors que les grands *Stenobrachiusnannochir* ont une vessie gazeuse recouverte de graisse. *Protomyctophum thompsoni* et *P. crockeri* conservent une vessie remplie de gaz.

Nous avons observé une haute teneur en lipides chez *S. leucopsarus*, *S. nannochir*, *L. ritteri* et *D. theta*, et une faible teneur dans les quatre autres espèces. Les Myctophidae à haute teneur en lipides ont un poids spécifique qui se rapproche de celui de l'eau de mer (1.026–1.030). *Tarletonbeania crenularis*, qui a une vessie gazeuse réduite, a un poids spécifique de 1.088. *Lampanyctus regalis* a un poids spécifique plus faible (1.040) à cause de la haute teneur en eau de ses tissus.

Le volume de la vessie gazeuse par rapport à celui du corps est en relation inverse de la taille et de la teneur en lipides de *S. leucopsarus* et de *D. theta*, ce qui démontre que les lipides assument graduellement la fonction primaire de flottabilité, à mesure que la vessie remplie de gaz s'atrophie avec l'âge. Ce changement peut éliminer les contraintes physiologiques qu'impose une vessie remplie de gaz, et permet des déplacements nycthéméraux plus étendus chez les adultes.

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MYCTOPHIDS have an euphysoclistous swimbladder which is often gas-filled (Marshall 1960). Because they have gas inclusions that may effectively scatter and resonate sound and because they are common oceanic animals that are known to undertake diel vertical migrations, myctophids have been considered sources of scattering layers in the ocean (Marshall 1951, 1960; Tucker 1951; Hersey and Backus 1961; Taylor 1968).

The two most common myctophids (lanternfishes) off Oregon, *Stenobrachius leucopsarus* and *Diaphus theta*, are known to migrate vertically within 12 kHz scattering layer depths (Pearcy and Laurs 1966; Pearcy 1964; Pearcy and Mesecar 1971). These species were reported to lack a gas-filled swimbladder (Ray 1950; Jollie MS 1954; Barham MS 1956), but Capen (1967) found that small specimens of *S. leucopsarus* had gas-filled swimbladders and suspected that small specimens of *D. theta* also may have gas in their swimbladders. Thus, adults of these abundant species, lacking gas-filled swimbladders, may not scatter sound as effectively as juveniles of these species or other less common species with gas-filled swimbladders.

This study was conducted on the morphology of swimbladders to learn which species and sizes of myctophids off Oregon contain gas-filled swimbladders and which species lack gas-filled swimbladders. Such knowledge is needed to correlate the depth distribution of effective sound scattering organisms collected in nets with the depths of sonic scattering layers. Lipids and specific gravity were also studied because of their interrelation with swimbladders and buoyancy.

Materials and Methods

Fishes were collected off Oregon with an Isaacs-Kidd midwater trawl equipped with a 5-mm mesh liner and a ½ m diameter codend of a 0.571 mm mesh. Preserved specimens were fixed at sea in 10% formalin and stored in 36% isopropyl alcohol. Standard length was measured to the nearest millimeter.

Swimbladder volume was studied in 123 preserved specimens of eight species of myctophids. The major and minor external axes of the swimbladder were measured with an ocular micrometer and a stereo dissecting scope. For collapsed swimbladders, one half of the circumference was measured. Total swimbladder volumes were estimated by the formula for an oblate spheroid, $V = 4/3\pi ab^2$ (Capen 1967), where a is the minor and b the major axis. Total body volume was estimated by weighing individual fish in air and suspended in distilled water, and estimating the volume of displaced water.

For study of their structure swimbladders selected from 46 fish were embedded in paraffin, sectioned, and stained with Mallory's triple stain or hemotoxin and eosin. Certain fat-invested swimbladders were sectioned with a cryostat and stained with Sudan IV for lipids.

To determine whether or not swimbladders contained gas, fresh specimens were collected at night in midwater trawls in the upper 50 m. Fish were dissected under water and the swimbladder was punctured to release any gas bubbles. The presence of gas in the body cavity was considered evidence for gas in the swimbladder since the swimbladder may have ruptured during the trawl ascent. One hundred thirty-nine specimens of eight species were examined in this manner.

In addition, 206 frozen specimens were analyzed for lipid content after they were dried to a constant weight at 70 C. Lipids were extracted with a two to one mixture of chloroform and methanol in a Soxhlet extractor, then each specimen was dried and reweighed. Lipid content was calculated by difference. Small individuals of the same size were grouped to provide a larger biomass. Seventy-eight extractions were made in this manner.

Specific gravity was estimated by two methods. In the laboratory intact frozen specimens were weighed in air and in distilled water, and at sea fresh specimens were suspended in a series of gum arabic solutions of known densities. Care was taken to exclude air from the gill chamber during all specific gravity measurements.

Types of Swimbladders

The myctophids were found to have three types of swimbladders. Thin-walled, ellipsoid swimbladders (Fig. 1), were found in all small specimens of all species studied. As the fishes grow, the thin-walled swimbladder may be unmodified, or it may become fat-invested in which case the gas phase is replaced by fatty tissue (Fig. 2), or the swimbladder may be reduced in size and have thick walls (Fig. 3).

The swimbladders of *Stenobrachius leucopsarus* and *S. nannochir* becomes fat-invested with age. Fatty reticular tissue on the anterior and posterior ends of the swimbladder in small specimens (Fig. 2) stains red with Sudan IV in frozen sections indicating the presence of lipids. The reticular structure is continuous with a layer of the swimbladder wall and is bounded by the outer layer, indicating that the fatty cells form inside the wall of the swimbladder, not outside as described by Capen (1967). As the fish grows larger, the fatty tissue increases and the size of the swimbladder is reduced until only a tube of fatty tissue remains in large individuals. The volume of the entire swimbladder decreased from about 4–5% of total body volume in small specimens of 20 mm to about 0.4% in large specimens (>70 mm) of *S. leucopsarus* (Fig. 4A) and also in *S. nannochir*.

The swimbladders of *Lampanyctus ritteri* and *L. regalis* regress with increasing size of fish but, unlike *Stenobrachius* spp., they do not become invested with fat. The volume of the swimbladder decreased from about 2% of body volume in small individuals (<25 mm) to 0.01% or less in large individuals (>80 mm). The swimbladder wall is

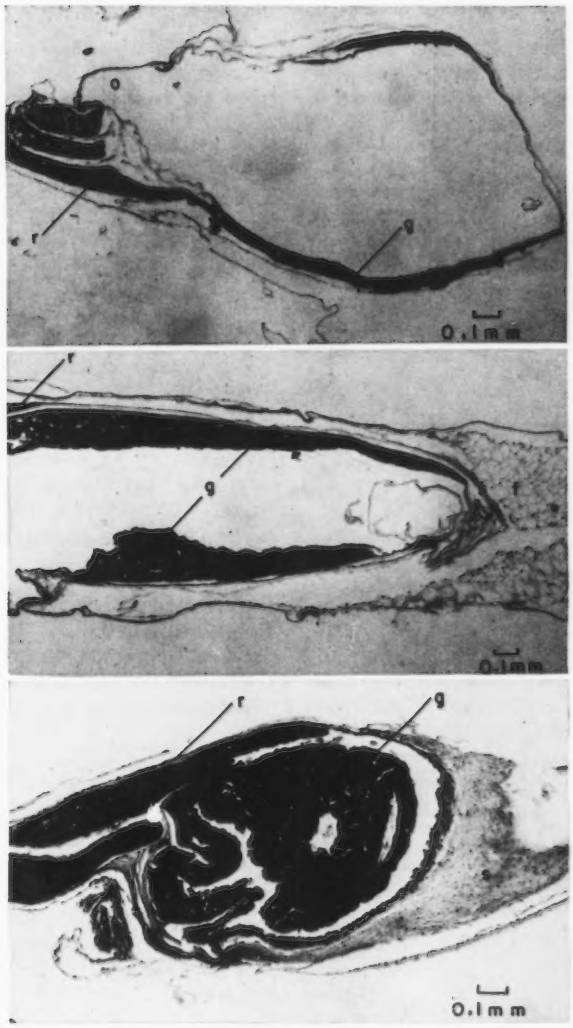

FIG. 1. (*Top*) Swimbladder of *Diaphus theta*, 23 mm standard length. g = gas gland, o = oval, r = rete mirabile.

FIG. 2. (*Center*) Fat-invested swimbladder of *Stenobrachius leucopsarus*, 36 mm. f = fat-filled tissue, g = gas gland, r = rete mirabile, s = submucosa.

FIG. 3. (*Bottom*) A reduced swimbladder of *Diaphus theta*, 38 mm. g = gas gland, s = submucosa, r = rete mirabile.

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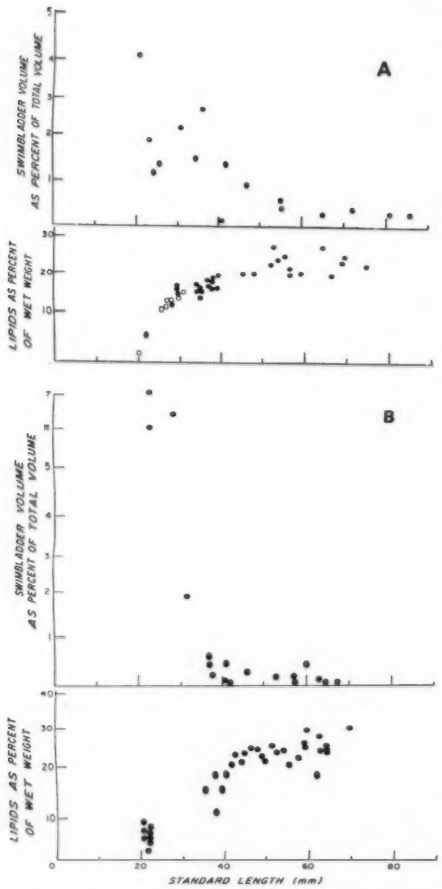
A
SWIMBLADDER VOLUME AS PERCENT OF TOTAL VOLUME
LIPIDS AS PERCENT OF WET WEIGHT
B
SWIMBLADDER VOLUME AS PERCENT OF TOTAL VOLUME
LIPIDS AS PERCENT OF WET WEIGHT
STANDARD LENGTH (mm)

FIG. 4. Swimbladder volume and lipid content versus length of *Stenobrachius leucopsarus* (A) and *Diaphus theta* (B).

thickened with connective tissue and the lumen is occluded by the gas gland in large individuals.

The swimbladders of *Diaphus theta* do not grow proportionally with the rest of the fish and are sometimes reduced in large specimens. The swimbladder volume in small individuals was about 6–7% of the body volume (Fig. 4B). In large specimens (>25 mm) the swimbladder volume averaged only 0.3% of body volume. There was considerable variability in the large specimens, however. Some had reduced, thick-walled swimbladders with the lumen occluded by the gas gland (Fig. 3), but other adults of the same size had small, thin-walled swimbladders that were not occluded.

Swimbladders of large *Tarletonbeania crenularis*, like *D. theta*, also have two distinct forms. Small, reduced swimbladders were present in some specimens while thin-walled, capacious swimbladders were present in others. The swimbladder to body volume was 0.5% or less in reduced forms and greater than 6% in thin-walled forms. Thin-walled swimbladders predominated (9 of 13) in specimens less than 40 mm; reduced swimbladders predominated (15 of 20) in larger specimens.

All sizes of both *Protomyctophum thompsoni* and *P. crockeri* had thin-walled swimbladders. However, estimated volumes of the swimbladders in both species varied, irrespective of size, from 5.5% to less than 1.0% of the body volume. The swimbladders appeared collapsed in those specimens with small volumes, but the lumen was not occluded by an enlarged gas gland as in the reduced swimbladders of the other species.

Gas was found in small *S. leucopsarus* and *L. regalis*, in both small and large *D. theta* and *T. crenularis*, in large *P. thompsoni*, and in small *P. crockeri* (Table 1). All of the gas-filled swimbladders had thin walls. Gas was never found in the fat-invested swimbladders of large *S. leucopsarus* or in the reduced swimbladders of large *L. ritteri*. Some of the large *T. crenularis* and *D. theta* had gas inclusions; but other individuals of both species had reduced swimbladders without gas. Thus, the presence of gas in thin-walled swimbladders and the absence of gas in fat-invested or reduced swimbladders corroborated our findings based on the morphology of preserved specimens. The absence of gas in large *S. nannochir* and *L. regalis* and the presence of gas in small *P. thompsoni* and large *P. crockeri* are inferred on the basis of the swimbladder morphology and the presence or absence of gas found in their congeners.

Lypid Contents and Specific Gravities

In large individuals the highest fat content occurred mainly in those species which lack a gas-filled swimbladder as adults, i.e. *S. leucopsarus*, *S. nannochir*, and *L. ritteri*; the lowest fat content occurred mainly in species which retain gas-filled swimbladders, i.e. *P. thompsoni* and *P. crockeri*. Exceptions were *D. theta*, which has either a small gas-filled swimbladder or a reduced swimbladder, but a high fat content; *T. crenularis*, which has either a gas-filled or a reduced swimbladder, but a low lipid content; and *L. regalis*, which has a reduced swimbladder and a low fat content.

TABLE 1. Occurrence of swimbladder gas in fresh myctophids.

Species	Gas present		Gas absent	
	No.	Length (*mm*)	No.	Length (*mm*)
Stenobrachius leucopsarus	27 S[a]	22–39	5 L[b]	48–72
S. nannochir	S	<35[c]		L>35[c]
Lampanyctus ritteri	S	<50[c]	8 L	50–120
L. regalis	1S	<50		L>50[c]
Diaphus theta	11 S	17–22	2 S	22–24
	9 L	34–62	30 L	29–70
Tarletonbeania crenularis	9 S	23–33	5 S	29–34
	4 L	30–47	15 L	36–70
Protomyctophum thompsoni	S	<35[c]		
	13 L	35–50		
P. crockeri	2 S	19–26		
	L	>30[c]		

[a]Small individuals.
[b]Large individuals.
[c]Inferred from morphology.

The total lipid contents of *S. leucopsarus* and *D. theta* were directly related to the size of the individual (Figs. 4A and B). The lipid content of small individuals (<25 mm) was less than 10% of wet weight. Large individuals (>30 mm) had a more variable lipid content which averaged 18.7 and 22.1% of wet weight, respectively (Table 2). Hence, lipid content and swimbladder volume were inversely related in these species (Fig. 4A and B), suggesting that lipids assume the buoyancy function of the gas-filled swimbladder as it regresses with age.

The values for lipid content of *T. crenularis* were plotted against fish size, but the slope of a regression line for 22 specimens (22–70 mm) did not differ significantly from zero ($t = 0.4$, 21 df). Moreover, the mean lipid contents of fish with gas and fish without gas were not significantly different ($t = 0.4$, 15 df). Apparently, lipids do not have a major role in buoyancy of this species even when the swimbladder is reduced.

The specific gravities of large *S. leucopsarus*, *S. nannochir*, *L. ritteri*, and *D. theta* that were frozen at sea and weighed in air and water were near the density of sea water (1.026–1.030) (Table 2). All the specimens of these sizes had fatinvested or reduced swimbladders without a gas phase. All of the specimens lost scales during capture so live animals may be denser than these results indicate.

Estimates of specific gravity of fresh specimens with gum arabic solutions were similar to those based on weights. The specific gravities of 17 fresh *S. leucopsarus* with gas and three without gas were less than 1.030, and those of two specimens with gas and two without gas were between 1.030 and 1.042. Those of three fresh *L. ritteri* were between 1.030 and 1.048. Specific gravity estimates of individuals which had gas-filled swimbladders are of questionable value since gas could either expand during ascent from depths of capture or escape from ruptured swimbladders.

Thawed specimens of *T. crenularis* were considerably denser than sea water (1.088, Table 2), as were seven fresh specimens without gas-filled swimbladders (1.086). The average specific gravity of eleven fresh specimens with gas-filled swimbladders was lower (1.056), but the expansion of gas during ascent from depth of capture may have produced these low values. We conclude from these results that without a gas-filled swimbladder *T. crenularis* would have to swim constantly to maintain position.

Although the specific gravity of a fresh specimen of *L. regalis* with gas (50 mm) was less than 1.030, large thawed specimens without gas (Table 2) were denser than seawater but not nearly as dense as *T. crenularis*. The body was flaccid and contained 86% water (on a weight basis) compared to 72% in *L. ritteri*. *Lampanyctus regalis* is a lower mesopelagic species (Pearcy 1964) that appears to have achieved neutral buoyancy in the same way as bathypelagic fishes by reduction of skeletal and muscular tissues (Denton and Marshall 1958; Marshall 1960).

Discussion

The swimbladders found in all juvenile myctophids, in the adults of *P. thompsoni*, and *P. crockeri*, and in some adults of *D. theta* and *T. crenularis* were thin-walled and gas-filled, morphologically similar to those that Marshall (1960) characterized for the family.

The situation in *D. theta* and *T. crenularis*, in which both thin-walled and reduced swimbladders occurred, is curious. Capen (1967) also noted two types of swimbladders in *D. theta* but did not find gas in fresh specimens. We found no relation between type of swimbladder in these species and season of the year or sex of individuals. Thus, the ecological significance of this biphasic swimbladder system in the adults of these species is unknown. Conceivably fishes with different swim-

TABLE 2. Average specific gravities of thawed myctophids frozen at sea. All individuals lacked gas-filled swimbladders.

Species	No.	Standard Length (mm)	Specific Gravity
S. leucopsarus	5	58–80	1.027(1.025–1.031)[a]
S. nannochir	2	90–102	1.026
L. ritteri	2	73–90	1.026(1.023–1.030)
D. theta	4	40–64	1.038(1.025–1.062)
L. regalis	2	84–85	1.040(1.040–1.041)
T. crenularis	2	41–59	1.088(1.088–1.089)

[a]Range.

bladder types could have different patterns of vertical migration and behavior.

Fat-invested swimbladders were found in large *S. leucopsarus*, confirming the earlier findings of Capen (1967), and in *S. nannochir*. Reduced swimbladders occurred in all large *L. ritteri* and *L. regalis* and in those *D. theta* and *T. crenularis* which lacked thin-walled swimbladders. In general, body lipid content was high in fishes that lacked gas-filled swimbladders (with the exception of *L. regalis*). Similarly, Nevenzel et al. (1969) found larger amounts of lipids in *L. ritteri*, *S. leucopsarus*, and *D. theta* than in *T. crenularis*.

Specific gravity measurements indicate that those fishes with high lipid content (*S. leucopsarus*, *S. nannochir*, *D. theta*, and *L. ritteri*) approach neutral buoyancy. Yet the observed lipid content is not as great as Taylor (1921) predicted for neutral buoyancy. His theoretical value, based on specific gravities of 1.076 for fat-free tissue and 0.925 for lipids (cod liver oil), is 29.23% of the wet weight. Our highest observed lipid value, however, was only 22.1% of wet weight (Table 3). Thus a lower tissue density and/or lipid specific gravity is required to explain the low specific gravity of these fishes.

The lipids found in myctophids may, in fact, be lighter than Taylor's value. Nevenzel et al. (1969) reported waxy esters (chain length 30–38) as the principal lipids in *S. leucopsarus*, *L. ritteri*, and *D. theta*. These lipids may be less dense than cod liver oil, which is composed of lipids with chain lengths of 18–20 (Morrison and Boyd 1966). Nevenzel et al. (1966) cite a specific gravity of 0.86 for oleyl oleate, a wax ester. Using this value for lipids and 1.076 for fat-free tissue, a lipid content of 19.4% of wet weight produces a specific gravity of 1.026. This value compares favorably with the observed lipid contents (Table 2) for *S. leucopsarus*, *S. nannochir*, *D. theta*, and *L. ritteri*. Thus low-density lipids may provide neutral buoyancy for these species. Lee et al. (1971) found that triglycerides were metabolized rapidly while wax esters were utilized more slowly in starved *Gaussia princeps* (Scott), a large mesopelagic copepod. Accumulating wax esters may have two advantages over triglycerides: a stable buoyancy fraction and a long-term energy reserve.

TABLE 3. Average lipid contents of large individuals of the various myctophids.

Species	No.	% Wet Wt.	% Dry Wt.
D. theta (> 35 mm)	28	22.1±0.7	63.6±1.2
S. leucopsarus (> 30 mm)	28	18.7±4.0[a]	59.8±4.0
S. nannochir (> 85 mm)	5	18.9±2.5	56.8±1.0
L. ritteri (> 58 mm)	10	16.0±0.3	57.8±2.0
P. thompsoni (> 25 mm)	19	4.9±0.37	24.1±1.0
P. crockeri (23 mm)	2	4.2	19.2
T. crenularis (> 23 mm)	25	4.2±0.2	19.2±0.8
L. regalis (> 50 mm)	5	2.2±0.7	16.6±4.6

[a]One standard deviation of mean.

The presence of a gas phase in swimbladders is related to the vertical distribution and migration of myctophids and their importance in producing sound scattering layers in the ocean. Of the four common myctophids that Taylor (1968) caught in his study off British Columbia, *S. leucopsarus* and *D. theta*, species with fat-invested swimbladders as adults, made the most extensive vertical migrations and were most numerous below the main sonic scattering layer during the day. *Protomyctophum thompsoni*, a species with a gas-filled swimbladder, made less extensive migrations and was often found at scattering layer depths. Barham (1971) observed from submersible dives that immature myctophids, with potentially resonant gas-filled swimbladders, are sometimes concentrated at scattering layer depths while large individuals were concentrated at deeper levels. Studies with opening–closing nets (W. G. Pearcy unpublished data) indicated that large *S. leucopsarus*, without gas-filled swimbladders, have a greater vertical range of diel migration than juveniles, which have gas-filled swimbladders. However, small *S. leucopsarus* were also found in a 350–420 m scattering layer during both day and night periods, indicating that some juveniles of this species probably migrate little if at all. (Pearcy and Mesecar 1971).

Maintaining a constant volume of gas in a swimbladder during rapid, extensive vertical migrations is no mean physiological feat. It requires impressive capabilities of gas secretion and resorption (Jones 1951, 1952; Kanwisher and Ebeling 1957; D'Aoust 1971; Alexander 1971). These constraints may limit vertical movements of fishes with gas-filled swimbladders. Therefore replacement of gas by lipids as the main buoyancy regulator, as in large *S. leucopsarus* and *D. theta*, may permit more extensive vertical migrations in these myctophids.

Acknowledgments

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A Quantitative Evaluation of Survival to the Adult Stage and Other Characteristics of Pink Salmon (*Oncorhynchus gorbuscha*) Produced by a Revised Hatchery Method Which Simulates Optimal Natural Conditions

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BAMS, R. A. 1972. A quantitative evaluation of survival to the adult stage and other characteristics of pink salmon (*Oncorhynchus gorbuscha*) produced by a revised hatchery method which simulates optimal natural conditions. J. Fish. Res. Bd. Canada 29: 1151–1167.

To evaluate a hatchery method utilizing a gravel medium and filtered water during incubation, comparisons were made between naturally and artificially propagated parts of one native stock of pink salmon (*Oncorhynchus gorbuscha*) of the Tsolum River, *B.C.* Yolk utilization (growth rate) of fry was unimpeded in the hatchery environment, but hatchery fry migrated about 6 days earlier than creek fry. Hatchery survival was about six times that in the creek. The main test criterion — relative survival to the adult stage of fish from both treatments following migration from the creek — was tested by release of two similar groups of fry identified by a double finclip and recovery of marked adults upon return. Both groups survived at virtually the same rate. Numbers of actual mark recoveries were adequate to demonstrate statistically that survival of hatchery fish following release was not less than 90% of creek fry survival and that the final gain ratio of the hatchery treatment was not less than 5.44 ($P < .005$). Adult size, sex ratio, fecundity, and timing were not adversely affected by the hatchery treatment.

BAMS, R. A. 1972. A quantitative evaluation of survival to the adults tage and other characteristics of pink salmon (*Oncorhynchus gorbuscha*) produced by a revised hatchery method which simulates optimal natural conditions. J. Fish. Res. Bd. Canada 29: 1151–1167.

En vue d'évaluer une méthode de pisciculture utilisant un milieu de gravier et une eau filtrée, l'auteur compare, à même une population indigène de saumons roses de la rivière Tsolum, Colombie-Britannique, un groupe propagé naturellement avec un autre propagé artificiellement.

La résorption du vitellus (rythme de croissance) n'est aucunement entravée en milieu de pisciculture, mais les alevins ainsi produits émigrent environ 6 jours plus tôt que ceux du ruisseau. La survie en pisciculture est à peu près 6 fois plus élevée qu'en milieu naturel. Le principal critère — survie relative des deux groupes après avoir quitté le ruisseau — a été mesuré en libérant un nombre égal d'alevins des deux groupes, identifiés par ablation de nageoires, et en recapturant les adultes au retour. Le taux de survie a été pratiquement le même pour les deux groupes. Le nombre de recaptures a été suffisant pour permettre à l'auteur de prouver statistiquement que la survie des saumons de pisciculture après libération n'est pas moins de 90% de celle des alevins de ruisseau, et que l'indice de gain final résultant du traitement en pisciculture n'est pas moins de 5.44 ($P < .005$). Enfin, le traitement n'a pas d'effets adverses sur la taille adulte, la proportion des sexes, la fécondité et l'époque du retour.

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NATURAL production of pink and chum salmon from potential egg deposition to migrating fry shows a general range in survival from 5 to 20%, occasionally larger. Hatchery methods are able to greatly improve survival over this period and average gains from 4 to 10 times over natural production are routinely producible by preventing high larval mortality. Increased egg to fry survival in hatcheries has not resulted in similarly increased egg to adult survivals. In North America, attempts at raising pink and chum salmon in hatcheries have been limited and were almost invariably confounded with transplanting brood stock from donor streams often hundreds of miles away. Evaluations to the adult

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stage were inadequate and indications are that a few continuing returns have been achieved only when the fry were held and fed prior to release from the rearing facility (Hood Canal trials by the Washington State Department of Fisheries; Noble MS 1964; Anon. 1968 et seq). Adult returns to an exe perimental pink salmon hatchery, operated by th- Fisheries Research Board of Canada from 1956 to 1961 on the Skeena River, were far below average natural production in the area on an egg to adult survival basis, although substantial gains were achieved at the fry stage.

My research into biological requirements of larval salmon disclosed several factors previously not, or inadequately, recognized as conditions essential to the production of viable fry. Reduced growth and subsequent loss of potential size during the larval period were caused primarily by suboptimal conditions of metabolites and silt in the incubational environment and a lack of supporting substrates to satisfy the innate righting response (Bams 1969). Reduced size at emergence depressed swimming performance and increased vulnerability to predation, factors considered indicative of reduced survival potential (Bams 1967).

On the basis of the newly discovered and already known requirements a new hatchery method was devised. A first pilot operation was carried out during 1967–68 at Hooknose Creek, King Island, B. C., on pink and chum salmon and demonstrated that growth rate (yolk utilization) and rate of development in the hatchery environment equalled that observed in the stream (Bams 1970). Subsequently, a series of tests was designed to evaluate the new method at both the migrant fry and the returning adult stages on consecutive year-classes of a stock of pink salmon. Pink salmon were chosen for the test because of the invariable age at return and the short life cycle.

The ultimate test of an enhancement method spans the entire life cycle. The main test criterion of these evaluation tests was based on the relative survival rates of fish produced naturally and artificially, as expressed in the change observed in the ratio in abundance of marked populations at time os release and at return. No appreciable change in this ratio would constitute a complete success. Partial success would correspond with a partial retention of the original gain, a failure would be no gain or a loss in terms of survival of the naturally propagated population. This paper gives the results of the first test in the series and is of particular importance because it is unique in respect to genetic composition of the stock used: it is the only test in the series of which the control was natural, wild stock. Subsequent tests will be carried out on increasingly admixed hatchery and wild stocks and induced genetic effects may influence results.

The results of this experiment are of general importance where they bear on an assumption regarding selectivity of mortality in the creek environment, an assumption basic to all artificial propagation methods attempting to increase egg to fry survival rates. My earlier field observations led to the belief that a significant proportion of creek mortality was nonselective for quality of brood, but was associated with location of the redds and the vagaries of intergravel water supply in a changing creek environment. The experiment here presented is a test of this hypothesis because success of the hatchery method, as expressed in survival to the adult stage, can only occur if larval mortality in the stream is not a clear function of quality and is not correlated with survival following emergence. Put differently, the classical criticism that hatcheries produce higher egg to fry survivals by merely delaying mortality of inferior individuals that normally die in the creek environment is refuted when a hatchery can produce seemingly normal fry at greatly increased survival rates that carry through to the adult stage.

Methods

The test was carried out on the native stock of pink salmon of the Tsolum River, Comox Bay, B.C. (Fig. 1).

The hatchery facilities were built on a tributary, Headquarters Creek, near its confluence with the Tsolum. The water supply came directly from the creek by means of a well connected to the creek with an open pipe.[1] Water was pumped by one of two electric pumps, or an automatic-start diesel standby pump, to the hatchery building, passing through a sand filter removing particles of 5 μ and over, and discharging into a constant-level head tank of about 2900-liter capacity. Hydraulic lift to the pumps was 3.5 m and static head from the pumps 6.8 m. Nine stacks of eight-tray Heath incubators contained the brood to the eyed stage. The brood was then transferred to four 4 × 8 × 4 ft high gravel boxes, equipped with an upwelling water supply of approximately 35 liters/min, leak-proof liners of non-toxic formula ABS plastic, and containing a gravel medium of crushed rock of commercially available size range 0.75–1.25 inches. Crushed rock is used because of its favorable crevice-to-solid ratio and superior support surface qualities. A 15-kva, diesel-driven generator supplied emergency power.

Temperatures of creek and hatchery water were recorded twice daily at about 0800 and 1700 hr. These recording times fall close to average daily minimum and maximum temperatures. Based on comparisons with

[1]This open system has since been changed to a much more satisfactory Rainey-type intake, incorporating a perforated pipe in a bed of gravel underneath the stream.

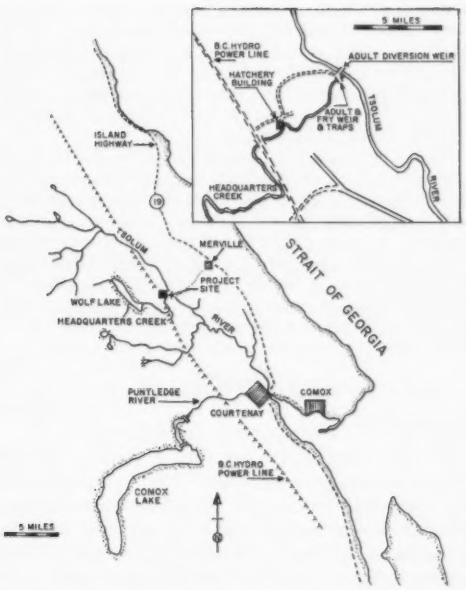

FIG. 1. Map of geographical area and site of the project.

more accurate time–temperature integrations calculated on hourly intervals, accuracy of this method is estimated to be about ±5%.

Oxygen determinations of the water percolating from the gravel in the boxes at time of highest demand, i.e. immediately prior to fry out-migration, showed a drop in O_2 concentration of from 1.5 to 2.0 ppm from near saturation at about 12 ppm.

A two-way fish weir was constructed at the mouth of the creek, 520 m downstream from the hatchery, which could trap all ascending adult fish or descending fish. A floating, inclined-plane trap upstream of the weir provided an index of daily fry abundance and was used on a few occasions to estimate daily outputs of creek fry when the weir was inoperative due to high water levels.

Immediately above the confluence of the hatchery stream a diversion weir was installed across the Tsolum River to prevent fish from entering this part of the river, which was thought to be very unproductive due to extensive annual scouring of the stream bed.

To ensure comparability of the brood stock employed in both treatments, every sixth female taken from the weir trap was retained for hatchery purposes; the others were immediately released into the stream. For each female, one male was retained simultaneously without any selection for size or condition. Spawning routine was to strip five males into a spawning bucket, mix the sperm, add the eggs of five females by incision, and wash and water harden the fertilized eggs. Eggs were then placed in darkened Heath incubators, five females to a tray, and occasional treatments of malachite green were given at 5 ppm for 1 hr. When eyed, the eggs were picked, counted electronically (accuracy > 99.5%), and introduced in eight 3-inch layers to each of four gravel boxes. Flows in the incubators, at 10 liters/min, and, in the gravel boxes, at 35 liters/min, which was proportional to a design flow of 100 liters/min at 200,000 eggs per box, were virtually constant and checked daily.

Fry emerged at night and were trapped in troughs attached to the boxes. All hatchery and creek fry were counted in the morning following emergence and recorded for that day. Samples were taken a few fish at a time throughout the counting period on sampling days and preserved in 10% formalin in "whirl-pack" plastic bags. Samples were processed not less than 6 weeks after preservation, in order of capture, and in corresponding creek and hatchery pairs. Fork lengths (mm), and total wet weights (mg), were taken and stages of development were related to the factor K_D as follows: $K_D = \frac{10\sqrt[3]{W \text{ in mg}}}{L \text{ in mm}}$ (Bams 1970). Relations of mean fry lengths, weights, and stage of development in time proved to be curvilinear, and in addition to the usual arithmetic means of sample means, weighted means (escapements during period × sample means) were also calculated. Both grand means are given in the paper because variability in sample means and occasional very large weighting factors may have introduced error in the weighted means that would not occur in the arithmetic means. Neither type of grand means is fully satisfactory and both must be considered in evaluating the results. A third type of means, the total sample means, is of importance only to related statistics that cannot be calculated for the weighted data.

The evaluation program was designed and carried out as follows. Quantities of hatchery and wild fry were to be marked differentially by the removal of the adipose and one ventral fin (AdLV for hatchery, AdRV for wild) and released into the stream. Commercial catch and spawning escapement would be examined for marked fish. It was assumed that the symmetrical marks affect survival evenly. A quality control program throughout the marking period ensured that only "valid" marks were recorded for the test populations. Damaged fish and incomplete marks (one-half of fin left on) were omitted, good marks with one-eighth to one-twelfth of one fin left on were progressively discounted, and all perfect marks were counted. Discount rates were calculated from two to four times daily for each marker and applied to total production. Average rejection rates were low and similar in both sources. Where no correlation is known between our quality control criteria and subsequent recognition at the adult stage, survival rates may be biased, but the bias is the same for both sources and is thought to be small. The primary test was based on relative survival of the marked populations as expressed in the change observed in the ratio of the numbers at release and at return. To avoid absolute numbers hatchery success was expressed in the so-called "gain ratio," calculated as the quotient of the observed survival rate of hatchery fish over creek fish. A ratio greater than one, therefore, expresses gain; equal to one is zero gain; and less than one is a loss; all in terms of survival of the creek fish.

Three main factors were considered in designing the test: (1) comparability of the two test groups, (2) the number of marked adults that would have to be recovered to detect differences at preselected levels of significance, and (3) the number of fish needed in the return escapement to ensure continuation of the population.

Comparability of the two test groups was promoted by the choice of symmetrical finclip marks, by marking similar numbers of each source on each marking day, and by releasing all fry (both marked and unmarked) captured during the previous night simultaneously in the same location immediately after dusk. Because the two fry runs were out of phase, the maximum number that could be marked was determined by the smaller population available on any one marking day. Finclipping of each of the two groups was alternated between morning and afternoon, and treatment during counting, holding, and marking procedures was carefully kept the same. During the last 8 days of the marking period, 38,000 creek fry were held over for 1 or more days before being marked and released. Hatchery fry were not carried over in the same manner. This procedure may have introduced a bias. Changes in length, weight, and stage of development occurring over 1 or 2 days are highly insignificant when compared to the variability in each of these characteristics as measured in a migrant population of a single night. Where, furthermore, all other experimental fish had been delayed for 1 day, exposed to daylight, and formed schools prior to release, it is not likely that 1 or 2 additional days would introduce a bias serious enough to affect the test, but the event must stand recorded.

The second and third factors were solved by drawing up a schedule of expected survival rates and other pertinent values. The following values were adopted: escapement, 3000 fish; fecundity per fish, 500 eggs; creek survival, 0.15; sea survival both sources, 0.03; hatchery survival, 0.75; sea survival marked fish both sources, 0.015. Assuming a self-sustaining population prior to testing, it follows that fishing mortality could not exceed 0.5. To produce equal numbers of creek and hatchery fry the parent run would have to be split 5:1. To produce an escapement about equal to the parent run would require marking not more than 0.6 of each fry run. This would produce 1875 adults in the catch, and at a 50% recovery rate, 937 marks recovered from the fishery. To establish a 10% difference in survival at the 0.005 probability level some 800 recoveries would be required. The experiment, therefore, appeared feasible with the number of adult fish expected, and the marked fry populations would exceed the required minimum of about 45,000 each. The values established during the experiment varied considerably from the estimates, as will be demonstrated later.

Adult recoveries were made in fish packing plants at Namu, Steveston, and Vancouver. Emphasis was placed on loads arriving from areas producing marked fish, but other areas were continually monitored. Very few loads from preferred areas escaped inspection. Tallies were kept of the number of fish inspected. Marked individuals were processed to obtain hypural length, weight, sex, and mark(s), and some scales were also taken. The area of origin, date, and, when available, estimated total number of pink salmon in each load were recorded. Mark recoveries in the Tsolum River system were obtained mostly (96.7%) from the weir at Headquarters Creek. The remainder was collected during regular examination of spawning grounds below the weir. Adjacent streams were checked occasionally.

At the hatchery, fork length measurements on adult fish were obtained on dead fish before or after spawning. Weight measurements were obtained on brood stock only immediately before spawning. Egg counts were obtained on most of the brood stock in groups of five females mixed together; some single female counts were obtained to estimate variability. All females were checked following spawning and any eggs retained in the body cavity were added to the count.

Results

Parent Run

In 1968, 3500 adult pink salmon passed through the weir at Headquarters Creek; 2205 were males and 1295 females (sex ratio 1.70). Mean date of entry was, for the males, September 21, SD 5.4, and, for the females, September 20, SD 4.1. A total of 276 females was retained for hatchery propagation of which 13 died or contained green or damaged eggs. Average fork length of 351 males was 35.54 cm, SD 2.87, and of 281 females, 36.08 cm, SD 2.35. Average egg content of 263 females used in the hatchery was 1105. All fish were smaller and carried fewer eggs than is average for the species.

An estimated additional 1500 fish spawned in the main Tsolum. Most spawning occurred from 5 to 10 miles below the confluence with the hatchery stream. About a dozen small adults penetrated the diversion weir above the confluence and spawned in the upper Tsolum.

Eggs and Fry

Approximately 292,000 eggs were taken for hatchery propagation and an estimated 1.12×10^6 eggs were available for deposition in the creek (Table 1). Considerable superimposition of redds occurred in the creek and actual deposition was substantially less than potential. Egg retention in both hatchery and creek females was below 1% and is ignored in all calculations. A high early egg mortality occurred amongst the earlier spawnings. This was caused by siltation of the tray incubators following breakdowns in the water supply and the sand filter not yet being on-line. Survival in the gravel boxes (Table 1) was considerably higher amongst those eggs which had incurred a high initial mortality, indicating a "delayed mortality" of about 8% in the other two boxes in which the eggs had not been exposed to an earlier period of stress.

TABLE 1. Mean spawning and emergence days, egg and fry numbers, and survival percentages of hatchery and wild progeny 1968–69.

Source	Mean spawning day[a]	Total eggs	Mortality to planting	Survival G/E (%)[c]	Fry output	Mean emergence day	SD	Survival E/F (%)[c]	Survival G/F (%)[c]
Tank 1	Sept. 26	82,719	32,215	61.1	47,070	Apr. 15	4.6[d]	93.2	56.9
Tank 2	Sept. 26	75,301	24,220	67.8	46,592	" 16	3.8[d]	91.2	61.9
Tank 3	Oct. 3	64,911	3823	94.1	50,620	" 22	3.1	82.9	78.0
Tank 4	Oct. 3	69,195	4576	93.4	55,155	" 22	3.4	85.4	79.7
$\sum$ Hatchery	(Sept. 25)[b]	292,126	64,834	77.8	199,437	" 19	4.8	87.7	68.3
$\sum$ Creek	(Sept. 19)[b]	1.12×10^6	–	–	126,399	" 14	5.0	–	11.3

[a]This date was calculated for eggs surviving at time of planting.

[b]These are fence-entry dates; actual mean spawning date was 3 days later for the hatchery fish and is presumed to differ similarly for the creek fish.

[c]The survival percentages span three life stages: G = green egg stage; E = eyed egg (planting) stage; and F = emerged fry stage.

[d]These larger standard deviations were the result of the inclusion of some late eggs amongst mostly early spawned ones.

At mean time of egg deposition daily average temperatures in creek and hatchery were about 14 C, at time of emergence about 7 C. Total estimated degree-days (from 0 C) over the mean incubation periods were similar at 970 for the creek and 950 C degree-days for the hatchery. Temperature gain in the hatchery over the mean incubation period was estimated at about 40 C degree-days.

The creek fry began migrating earlier than the hatchery fry and maintained the lead throughout the run (Table 2; Fig. 2, 3). Mean emergence dates were: creek fry, April, 15.7, SD 5.0; hatchery fry, April, 19.3, SD 4.8. First, second (= median), and third quartiles of the runs were, respectively, April 11.7, 13.9, 16.6; and April 14.8, 19.2, 21.9. The creek fry, therefore, were ahead by 3.6 days according to mean dates, and by 5.3 days according to median dates. The rate of emergence was very fast; for the major part of the runs it was about 10.0%/day in the creek fry and 12.5%/day in the hatchery fry (Fig. 3, trend lines from about 10–80%).

TABLE 2. Weekly counts of creek and hatchery pink salmon fry, 1969.

Week ending	Creek	Hatchery
Mar. 15	163	14
22	208	30
29	654	24
Apr. 5	1882	70
12[a]	32,138	12,500
19[a]	74,829	83,994
26[a]	13,785	92,379
May 3	2569	8231
10	171	1986
17	–	209

[a]All marking occurred during these weeks.

Marking was carried out from April 9 to 26. An error of 2000 excess hatchery marks occurred on April 25 and could not be corrected because creek production had virtually ceased. After correction for incomplete marks and mortality prior to release, about 75,000 creek fry were released with a valid AdRV mark and 77,000 hatchery fry with a valid AdLV mark. In addition, approximately 120,000 unmarked hatchery and 50,000 unmarked creek fry were released.

Eyed egg to fry survival averaged 87.7% in the gravel boxes, which brought the green egg to fry survival to an average of 68.3%. Compared with the creek survival of 11.3%, the hatchery, therefore, showed an increase in survival rate of about six times and the gain ratio was 6.04.

Mean lengths of creek fry were consistently greater than those of hatchery fry (Table 3, Fig. 4). The weighted means differed by 0.70 mm, the means of eight common dates by 0.73. The latter difference was, for the Hooknose Creek pink salmon, 1.01 mm (Bams 1970).

Table 4 shows the results of nonparametric tests, which ignore possible heterogeneity in variance, on 16 samples paired by date. Significant differences between pairs are demonstrated during all but the tail end of the run, and between the totals.

The difference between the total sample means was 0.59 mm, with 99% probability limits of 0.40 and 0.78 (confidence limits test, Wilks 1949). Because the variances in the two sources are similar it is concluded that the difference in length between the two summed samples is statistically significant.

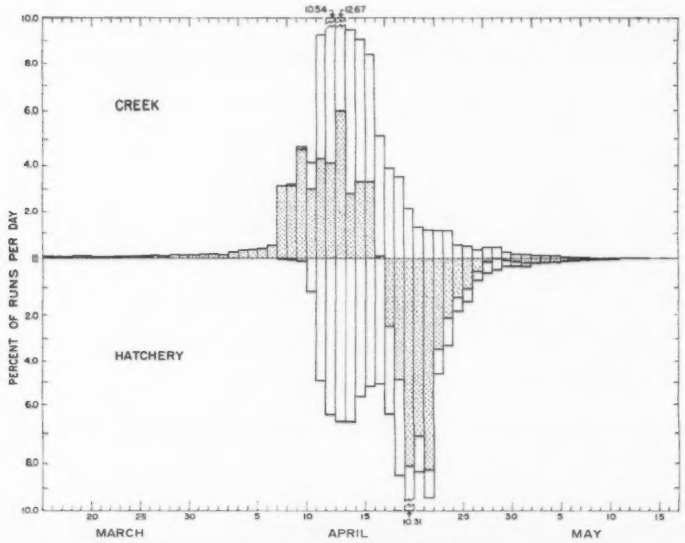

FIG. 2. Daily fry outputs from the hatchery and the stream, in percent of total for each run. The shaded areas indicate the daily difference in output between the two sources. Day numbering is from March 1 (= day 1).

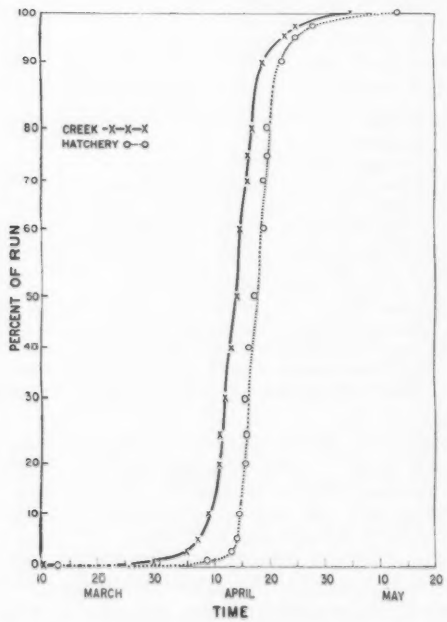

FIG. 3. Cumulative fry outputs from the hatchery and the stream in time. Trend lines, expressing rates of migration, were determined by eye.

By applying the same range limits to the difference between the more meaningful average means of the common dates, 0.73, we obtain a feducial range of 0.54–0.92. This is probably an overestimate because the limits were calculated on samples with a smaller difference between means.

Mean weights of hatchery fry were smaller than those of creek fry for the first part of the run, but similar for the remainder; the trend lines cross at about April 21 (Table 3, Fig.4). Total sample means differed by 9.7, the averaged means by 4.5, and the weighted means by 7.0 mg, the creek fry being the heavier. The 99% confidence limits of the difference in total sample means, 9.7, are 5.2–14.3, which makes this difference statistically significant. When these limits are applied to the average means difference, 4.5, insignificance is indicated at this level of probability. However, there is some doubt concerning the validity of this conclusion because the variances are dissimilar and the test is affected by both. The nonparametric Sign test also shows nonsignificance on the eight paired means. It is concluded that during the early part of the run the creek fry were significantly heavier than the hatchery fry (by not less than 5%) but that for most of the run there was no significant difference in weight.

Mean stage of development (K_D-index) was consistently more advanced (lower) in the creek fry (Tables 3, 4; Fig. 4). Differences between pairs of

TABLE 3. Lengths, weights, and developmental indices (K_D) of hatchery and creek pink salmon fry Headquarters Creek, 1969. (Symbols explained in footnote "a").

Date	S	N_s	N_w	Length (*mm*)			Weight (*mg*)			Developmental index		
				L_s	L_w	SD	W_s	W_w	SD	K_{D_s}	K_{D_w}	SD
3/13–19	1–4	25	29	29.15		1.209	196.2		19.58	1.992		.0705
3/20–26	1–4	22	22	29.98	*29.51*	1.447	210.8	*202.2*	16.51	1.986	*1.990*	.0895
3/27–2	1–2	50	54	31.05		1.494	234.2		29.06	1.982		.0609
"	3–4	9	9	29.29	*30.80*	1.480	211.7	*231.0*	25.25	2.033	*1.989*	.0551
4/3–5	1–2	50	70	31.23		1.386	235.1		24.24	1.975		.0607
"	3–4	7	7	29.54	*31.07*	2.166	210.4	*232.9*	28.69	2.013	*1.978*	.0957
4/6–15	1–2	50	50,871	32.20		1.099	240.6		22.40	1.930		.0519
"	3–4	50	937	31.60	*32.19*	1.217	234.1	*240.5*	26.69	1.948	*1.930*	.0455
4/17	1–2	50	28,995	32.65		1.041	240.2		21.59	1.902		.0503
"	3–4	50	15,640	32.19	*32.49*	1.075	248.0	*242.9*	24.88	1.949	*1.918*	.0375
4/22	1	48	3284	32.26		0.969	236.7		26.97	1.914		.0436
"	2	50	3182	32.43		1.020	225.9		21.95	1.876		.0431
"	3	50	25,607	32.33		0.984	240.2		21.18	1.921		.0343
"	4	50	22,062	32.13	*32.25*	1.027	237.7	*238.1*	24.95	1.925	*1.920*	.0398
4/25	1	50	2313	32.58		0.853	237.7		24.68	1.898		.0417
"	2	50	1851	32.07		0.953	226.3		22.89	1.897		.0394
"	3	50	15,382	32.23		1.022	240.2		24.50	1.926		.0378
"	4	50	18,700	32.51	*32.38*	0.921	253.4	*245.8*	22.00	1.944	*1.932*	.0381
4/29	1	50	965	33.09		0.933	223.6		23.20	1.831		.0466
"	2	50	719	32.78		1.169	225.2		26.10	1.852		.0432
"	3	50	2048	32.28		1.179	217.7		22.52	1.862		.0458
"	4	50	2686	32.21	*32.43*	0.991	217.5	*219.3*	22.52	1.864	*1.857*	.0441
5/3	1	48	590	32.62		0.889	221.3		21.66	1.851		.0471
"	2	50	418	32.64		0.998	220.5		25.17	1.848		.0474
"	3	50	882	31.96		1.217	220.9		24.90	1.888		.0398
"	4	50	1196	32.04	*32.21*	0.927	222.7	*221.6*	21.87	1.889	*1.876*	.0408
5/9	1	26	212	32.37		1.118	211.6		26.81	1.837		.0651
"	2	12	109	32.72		1.025	188.5		23.88	1.749		.0657
"	3	32	260	31.69		1.341	195.8		26.45	1.829		.0822
"	4	50	339	31.83	*32.02*	1.085	211.2	*204.3*	22.04	1.869	*1.836*	.0560
Total	Hatch	1279	119 K	32.08	*32.32*	1.300	228.0	*240.3*	27.20	1.903	*1.921*	.0693
Total (*m*)	"	8	–	31.93	–	–	232.7	–	–	1.925	–	–
3/26	Creek	50	1175	31.69		0.991	243.0		23.74	1.967		.0625
4/3	"	50	1732	31.80		1.007	248.7		26.93	1.975		.0403
4/10	"	50	32,138	33.69		1.022	255.9		25.94	1.882		.0363
4/17	"	50	69,881	32.78		1.128	246.1		27.56	1.908		.0421
4/22	"	50	15,269	32.93		1.106	243.0		24.85	1.892		.0466
4/25	"	50	3464	33.34		0.836	236.6		24.22	1.852		.0392
4/29	"	50	2228	32.76		1.202	220.4		36.00	1.837		.0524
5/3	"	44	512	32.32		1.323	204.2		32.51	1.816		.0376
Total	Creek	394	126 K	32.67	*33.02*	1.271	237.7	*247.3*	31.69	1.893	*1.898*	.0700
Total (*m*)	"	8	–	32.66	–	–	237.2	–	–	1.891	–	–
$\triangle_i$	H–C	$\sum_s$	$\sum_w$	–0.73	*–0.70*	–	–4.5	*–7.0*	–	0.034	*0.023*	–
$\triangle_i^1$	H–C	$\sum_s$	$\sum_w$	+0.12	*–0.12*	–	–	–	–	0.000	*0.000*	–
$\triangle_i$ HN	H–C	–	–	–1.01	–	–	–4.7	–	–	0.047	–	–
$\triangle_i^1$ HN	H–C	$\sum_s$	–	+0.17	–	–	–	–	–	*0.000*	–	–

[a]Symbols: Date, day or period of sample; Total (*m*), means for 8 paired dates on weighted parameters; S, source of sample; 1, 2, 3, 4: hatchery gravel boxes no. 1–4, in combined groups, or singly; N_s, number of fry in sample; N_w, total number of fry during sampling period; L_s, mean length of fish in sample; L_w, weighted mean length over period indicated; SD, standard deviation of sample; W and K_D, as for L; $\triangle_i$, difference between means of parameter as per samples; $\triangle_i^1$, difference between means of parameters adjusted for equal stage of development; HN, data from test at Hooknose Creek (see text).

samples were usually significant, the difference between the eight pairs of weighted means was significant ($P < .005$, Sign test), and the difference between the two total sample means was significant, the 99% confidence limits being 0.002 and 0.018. Here the significance applied to the difference between means only, because the variances were equal. It is concluded that the hatchery fry were consistently less advanced than the creek fry.

Returning Adults

Fish with clearly recognizable marks recovered from the canneries and the Tsolum River system are listed in Table 5. In the river most recoveries were made at the weir; only 21 marks were found below the weir, mostly immediately below the confluence of the hatchery stream with the main river. Although hundreds of fish in the lower river escaped inspection, spot sampling demonstrated a very low incidence of marked individuals amongst several hundred fish checked and the overall recovery rate in the river including the hatchery stream was estimated at better than 95%.

Spawning areas of three streams adjacent to the Tsolum River were checked for occurrence of marked pinks. Because only one male, AdLV, was recovered amongst hundreds of fish inspected,

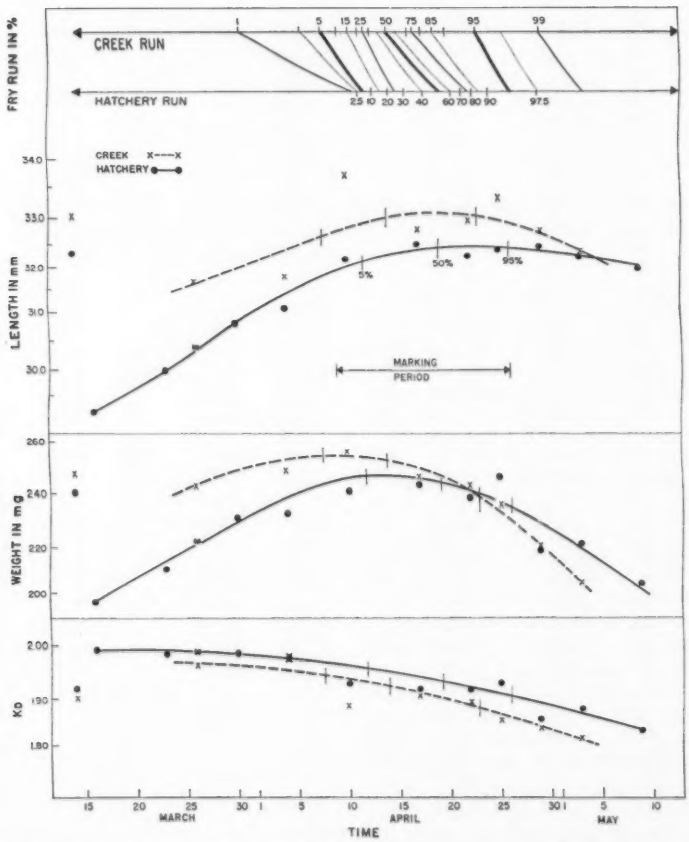

FIG. 4. Mean lengths, weights, and K_D-indices of samples of hatchery and creek fry on the dates indicated. Total weighted means for both sources are given on the far left. Trend lines were determined by eye and adjusted for agreement between length, weight, and corresponding K_D-values. Timing of both fry runs and the marking period are included. Data points in parentheses were calculated by appropriate weighting of adjacent points.

the incidence of Tsolum fish straying into neighboring rivers appeared to be negligible. Non-Tsolum fish straying into the Tsolum were of no concern to the main test, which depended solely on marked fish. The incidence of "naturally" occurring double marks was virtually nil, the occurrence of single ventral marks was 449 in 658,000, single adipose marks, 250 in 658,000 (Table 5), and, therefore, the chance of a natural double mark (Ad, either V) was on the order of 1 in 3.9×10^6.

The main evaluation test is based on the ratios of the numbers of fish in the two marked populations at the time of release and upon return. The observed and expected numbers of adults and the difference are:

No. fish	AdRV (creek)	AdLV (hatchery)	Total
Observed	584	582	1166
Expected	575.5	590.5	1166
Difference (%)	+0.75	−0.75	1.5

TABLE 4. Statistical analysis of paired samples of hatchery and creek pink salmon fry, Headquarters Creek, 1969.[a]

Sampling date	N_C[a]	N_H[b]	Length U'[c]	Length z[c]	Length P[d]	Weight U'[c]	Weight z[c]	Weight P[d]	Dev. index U'[c]	Dev. index z[c]	Dev. index P[d]
Mar. 26	50	81	1121.5	4.287	.000	1178.5	4.011	.000	1745.0	1.327	.092
Apr. 3	50	57	1003.0	2.639	.004	918.5	3.163	.001	1404.0	0.131	.448
10	50	100	339.0	5.917	.000	824.0	2.937	.002	564.0	4.729	.000
17	50	100	2016.5	1.931	.027	2367.0	0.530	.298	1836.5	2.645	.004
22	50	198	3243.5	3.772	.000	4019.0	2.054	.020	3996.5	2.104	.018
25	50	200	2178.0	6.183	.000	4817.5	0.399	.344	1296.0	8.099	.000
29	50	200	4542.0	1.003	.158	4902.0	0.214	.414	4103.5	1.960	.025
May 3	44	198	4184.0	0.410	.340	2442.5	4.557	.000	1567.0	6.640	.000
Total (Sign)[e]	8	8	–	0	.004	–	2	.145	–	0	.004

[a]Number of fry in creek sample.
[b]Number of fry in hatchery sample.
[c]Mann-Whitney U test statistics.
[d]One-tailed probability.
[e]Results of Sign test on weighted means for each date, where N = number of paired means available and $z = x$-statistic of Sign test.

TABLE 5. Recoveries of marked adult pink salmon from fish canneries and hatchery stream during 1970.

Date	Location	Fin marks observed[a] AdRV	AdLV	Ad	RV	LV	AdRVLV	RVLV	Approximate sample size	% load sampled
July 6–11	Vancouver	0	0	15	0	1	0	0	(10,000)[b]	–
12–18	Canneries	2	2	21	0	0	0	1	16,000	90.6
19–26	"	4	16	35	8	13	1	0	111,000	45.5
27–1	"	78	72	18	14	30	2	7	76,500	44.5
Aug. 2–9	"	115	136	14	29	39	2	8	111,000	57.3
10–16	"	95	112	37	36	49	4	9	103,000	54.2
17–23	"	4	13	21	18	21	0	5	(75,000)	42.8
24–29	"	1	5	14	16	17	0	5	55,500	55.4
Totals		299	356	175	121	170	9	35	(558,000)	50.2
July 7–Aug. 11	Namu cannery	11	14	75	75	83	0	7	(100,000)	27.7
Totals	All canneries	310	370	250	196	253	9	42	(658,000)	44.8
Sept. 10–Oct. 7	Tsolum system	274	212	17	12	22	1	1	4500	>95
Totals	All sources	584	582	267	208	275	10	43	–	–

[a]Ad, adipose; RV, right ventral; LV, left ventral fin.
[b]Figures in parentheses are gross approximations.

Chi-square analysis shows nonsignificance of the difference between observed and expected values ($\chi^2 = 0.248$, $P \simeq 0.6$). To establish what level of difference would be detected by the numbers of recoveries as obtained, χ^2 values were calculated for increasingly larger differentials in expected returns which, in effect, simulates increasingly poorer relative survival of the hatchery fish. The following table gives some critical values for minimum levels of difference detectable at the given levels of significance, assuming the survival of the hatchery fish to have been less than that of the naturally propagated fish:

Total difference (%)	χ^2	P
1.50	.248	.62
7.20	3.858	.05
9.00	6.672	.01
10.00	8.573	<.005
11.06	10.854	.001

The calculations demonstrate that the actual recoveries had one chance in 100 of being derived from a population in which the hatchery fish had survived less well than the creek fish by 9%, and only one chance in 1000 for a difference of 11%.

The actual return figures give net fry to adult survival rates, S, and instantaneous mortality rates, Z, for the marked hatchery fish, S = 0.76%, Z = 9.72, and for the marked creek fish, S = .78%, Z = 9.75. These figures correspond with a gain ratio at the adult stage of 5.97. Accepting a differential of 10% as the lower limit, the gain ratio could not have been less than 5.44 ($0.001 < P < 0.005$). Both figures compare favorably with the gain ratio at the time of emergence, 6.04.

A discrepancy is noted in the distribution of the returns. In the catch the hatchery fish outnumbered the creek fish, the reverse occurred in the escapement (Table 5, 6). Using Siegel's (1956) formula for testing a 2×2 contingency distribution, which is corrected for continuity, the difference proves highly significant, $\chi^2 = 12.77$, $P < .001$. As will be demonstrated in the Discussion, this result implies bias in the evaluation results given above. The bias favors the naturally produced population and is ignored here.

Of the fishery samples, 97% were processed to obtain information on sex, length, and weight. These data are listed with the creek data (Table 7). The fishery was selective for females: the sex ratio (male/female) was 0.73 in the hatchery fish and 0.70 in the creek fish, whereas reversed sex ratios occurred in the escapement.

The timing of the three adult runs into the hatchery stream (marked hatchery, marked creek, unmarked unknown) was similar with 1 day, or less, difference between the three groups in first and second quartile locations (Table 6). Creek males showed a slight but consistent tendency for earlier entry than hatchery males; unknown males, consisting of a mixture of hatchery and creek progeny, showed intermediate values (Fig. 5). In the females there was little or no difference between known hatchery and creek progeny, but unmarked fish were somewhat earlier. It is concluded that the hatchery treatment had little effect on time of entry of the progeny, and, particularly, on time of egg deposition.

As with the parent run, the males outnumbered the females (Table 6). The sex ratio was, for the known hatchery progeny, 1.87 ($\chi^2 = 17.85$, $P <$

TABLE 6. Fence entry records for marked hatchery (AdLV), marked wild (AdRV), and unmarked (NM) adult pink salmon, Headquarters Creek, 1970. Entry began September 10 and ended October 7.

Source	No. fish	Mean day of entry[a]	SD	Quartile 1	Quartile 2	Quartile 3	△[b] (Mn–Md)
AdLV ♂	127	19.22	5.87	14.97	17.25	24.17	1.97
AdRV ♂	160	16.98	3.48	15.00	16.26	18.23	.72
NM ♂	1784	18.91	5.42	15.45	17.57	22.69	1.34
Total ♂	2071						
AdLV ♀	68	19.91	5.05	16.10	18.50	23.50	1.41
AdRV ♀	110	19.48	4.64	16.23	18.58	22.93	.90
NM ♀	1079	18.57	4.47	15.93	17.49	22.00	1.08
Total ♀	1257						
Total	3328						

[a]September 1 = day one.
[b]Difference between mean and median days.

TABLE 7. Average hypural lengths, total wet weights, and egg contents of samples from three sources of adult pink salmon in Headquarters Creek and from two sources in the fishery. (Symbols explained in footnote "a")

Source	N	$\overline{L}$	SD	N	$\overline{W}$	SD	N	$\overline{F}$
C. AdLV ♂	30	39.44	3.76	30	1336	452	–	–
C. AdRV ♂	26	39.87	3.23	26	1383	431	–	–
C. NM ♂	165	40.15	3.62	165	1393	484	–	–
C. AdLV ♀	36	39.68	1.85	35	1262	224	37	1534
C. AdRV ♀	43	39.09 } *	1.71	42	1193	205	38	1423
C. NM ♀	136	39.64 } *	2.10	135	1251	224	125	1518
F. AdLV ♂	148	39.58	3.28	148	1500	443	–	–
F. AdRV ♂	123	39.51	3.29	123	1483	442	–	–
F. AdLV ♀	204	39.34	2.28	203	1437	278	–	–
F. AdRV ♀	176	39.25	2.19	175	1410	251	–	–

[a]Symbols: C, creekfish; F, fish caught in fishery; AdLV, marked hatchery; AdRV, marked creek; NM, unmarked unknown; N, number in sample; $\overline{L}$, mean hypural length in cm; $\overline{W}$, mean total wet weight in g; $\overline{F}$, mean number of eggs per female; SD, standard deviation. Confidence limits tests on differences between means not significant at $P = .1$, except * = $0.1 > P > .05$.

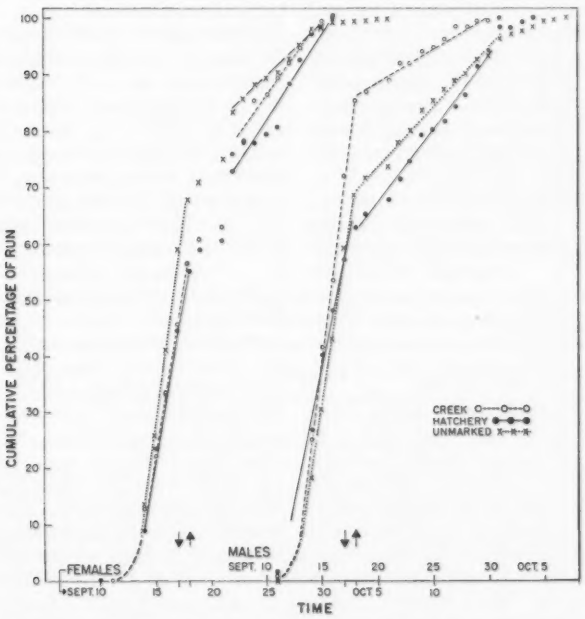

FIG. 5. Cumulative adult entries into weir trap in time. Runs are separated by sex and origin (marked hatchery, marked creek, and unmarked mixed stock). A break in mean entry rates is indicated by ↑, following a sharp reduction in river discharge effected at ↓.

.001); the known creek progeny, 1.46 ($\chi^2 = 9.26$, $.01 > P > .001$); and the unknown mixture intermediate at 1.65 ($\chi^2 = 174$, $P < .0001$). On first impression these data appear to indicate that the hatchery treatment favored male survival differentially. However, upon correction for selective mortality in the fishery, estimated sex ratios prior to fishing mortality were identical in the hatchery and creek adult populations (cf Discussion). Sex-ratio differences between the sources are statistically insignificant. For the known hatchery and creek returns, $\chi^2 = 1.41$, $.3 > P > .2$, and for the three sources, $\chi^2 = 1.72$, $.5 > P > .3$.

Table 7 shows that hatchery-produced males were somewhat smaller in length and weight than creek males. Unmarked males were slightly longer and heavier than those of the marked groups. Unmarked females were about the size of hatchery-produced females, and both were longer and heavier than creek females. All paired combinations (12) of samples, by sex, length, and weight, were tested for significance of difference between means (Wilks 1949). Creek females were marginally lighter than hatchery females, $0.1 > P > .05$; all other combinations were nonsignificant, $P > .1$. In adults obtained from the fishery, sample-sizes were larger, and differences in all paired combinations by sex, length, and weight proved nonsignificant, $P > .1$. It is concluded that the hatchery treatment had no measurable deleterious effect on the average hypural lengths and total wet weights of the returning adults.

Adults caught in the fishery were compared with those from the stream. The mean lengths were similar, both male and female comparisons being nonsignificant, $P > .1$. The mean weights, however, were consistently lower in the creek; the females had lost, in the hatchery stock 12.2%, in the creek stock 15.4%, and the males, respectively, 10.9% and 6.7% of total wet weight. Statistical significance of the differences (individual χ^2) was as follows: AdLV male, $.05 > P > .1$; AdRV male, $P > .1$; AdLV female, $P < .01$; AdRV female, $P < .01$. Average losses were: females, 13.8%; males, 8.9%. It is concluded that during the last 6 weeks prior to entering the spawning stream no change in hypural length occurred, but a loss in total wet weight was significant and was larger in the females than in the males. The latter observation may be related to differential changes occurring in body structure and volume and specific calorific value of sexual products.

The average fecundity of the females differed little between the three sources. The known wild females had the lowest count, the fish of mixed origin were higher by 6.7%, and the known hatchery females the highest with 7.8% more than the creek fish. Egg counts in salmon are directly correlated with size of fish and this relation is evident in the data (Table 7). However, in the much larger fishery sample no significant size difference occurred, and the phenomenon may, therefore, be a chance occurrence only. In any event, the hatchery treatment can be concluded not to have affected fecundity adversely.

Differences in variability are important from a genetic point of view. Table 8 shows that some differences in variance of length and weight between fish of hatchery and creek origin occurred. In the males no differences were evident in the fishery sample, but higher variances occurred in the hatchery fish in the river sample. The females showed higher values for fish of hatchery origin in both samples. Bartlett's tests indicate nonsignificance, $P > .05$ for all four groups, but the data are con-

TABLE 8. Variances of lengths and weights of adult male and female pink salmon caught in the creek and in the fishery separated by incubational origin.

Sex	Sample source	Origin[a]	Length			Weight		
			N	s^2[b]	Δ %[c]	N	s^2[b]	Δ %[c]
♂	Creek	H	30	14.130	+35.2	30	204.436	+9.9
"	"	C	26	10.455		26	186.024	
"	Fishery	H	148	10.752	-0.7	148	195.971	+0.1
"	"	C	123	10.825		123	195.806	
♀	Creek	H	36	3.408	+16.5	35	50.390	+19.4
"	"	C	43	2.925		42	42.200	
"	Fishery	H	204	5.186	+8.5	203	77.524	+23.3
"	"	C	176	4.781		175	62.875	

[a]Incubational origin: H, hatchery; C, creek; N, sample size.
[b]Variance of mean parameter.
[c]Difference in variance between H and C pair of samples, where C = 100%.

founded by differences in parameter means between creek and fishery samples. A Sign test on all eight paired-sample means shows significance, $P < .05$. It is concluded that variability in length and weight was the same or somewhat larger, particularly in the females, in the hatchery propagated stock than in the naturally propagated stock.

Survivals from Fry to Adult

The river entry records (Table 5, 6) showed total actual returns of 212 marked hatchery, 274 marked creek, and 2863 unmarked fish. These derived from, respectively, 77,000, 75,000, and 170,000 fry, and the corresponding survival rates, S, and instantaneous mortality rates, Z, to spawning were therefore, respectively: S = 0.28, 0.37, and 1.69%; Z = 5.90, 5.61, and 4.08. For the two marked sources combined, S_M = 0.32% and Z_M = 5.75. The difference between marked and unmarked fish therefore was: S_U/S_M = 5.26; Z_M/Z_U = 1.41.

Of the total loads handled at Vancouver and Namu canneries, 47 and 86%, respectively, contained information on both the total number of fish available in the load and the number actually sampled. Average percentages of the loads sampled were: Vancouver, 50.2%; Namu, 27.7% (Table 5). It is believed that very few loads containing our marked fish escaped inspection and also that the recovery rate of marked fish amongst those recorded as inspected was high. Therefore, an estimated total recovery rate of 0.50 appears reasonable and would definitely not overestimate the total catch. Actual mark recoveries in the fishery were 310 creek and 370 hatchery fish. At an estimated 50% mark recovery the estimated catch was therefore, respectively, 620 and 740 fish. Assuming the same ratio of marked to unmarked fish in the fishery as occurred in the river, 6.16, an estimated additional 8378 unmarked fish were caught in the fishery. At identical survival rates for hatchery and creek progeny and based on the numbers of unmarked fry released this group of fish consisted of 5948 (71%) hatchery and 2430 creek progeny. The contribution to the fishery was therefore 6688 hatchery and 3050 creek fish, for a total of 9738 fish. In the stream 4.3% of the marked fish were known to have spawned below the fence. Unmarked fish may have "strayed" similarly but it seems likely that some unmarked fish passed through the weir which had strayed in the opposite direction. No correction is therefore made for unmarked Headquarters Creek fish that may have spawned below the fence. This brings the total creek estimate to 3349 fish, the total run to 13,087, and the fishing mortality estimate to 74.4%. This figure is in good agreement with Department of Fisheries and Forestry (Vancouver) fishing-efficiency estimates of the area, which are from 75 to 80%.

On the basis of these estimates and the actual recoveries in the stream, total fry to adult survivals are estimated as follows:

Location	Marked fish	Unmarked fish
	Hatchery	
Catch	740	5948
Escapement	212	2033
Total	952	7981
From fry	77,000, S = 1.24% Z = 4.39	120,000, S = 6.65% Z = 2.71
	Creek	
Catch	620	2430
Escapement	274	830
Total	894	3260
From fry	75,000, S = 1.19% Z = 4.43	50,000, S = 6.52% Z = 2.73

Survivals from Egg to Adult

The egg to adult survival figures based on the estimates given in the previous section are for the unmarked fish: for hatchery, 4.49% (Z = 3.10) and for creek, 0.73% (Z = 4.92). The gain ratio of these survival figures is 6.16. For the marked fish the figures are: for hatchery, 0.83% (Z = 4.79) and for creek, 0.13% (Z = 6.62); the gain ratio is 6.27. These figures are somewhat higher than the estimates given on p. 1160, which were derived from the actual returns only, whereas these figures are based on the expanded results. This procedure capitalizes on the favorable ratio of hatchery to creek returns as this occurred in the fishery. The validity of the increase is not in doubt, because it was shown (p. 1160) that the discrepancy in the ratios at return was highly significant.

Discussion

Evaluation at the Fry Stage

To obtain and compare mean developmental rates and mean lengths of incubational periods, stages of development, times of emergence, mean spawning dates, and approximate mean temperatures during spawning and migration must be interrelated, as was done for the Hooknose Creek data (Bams 1970). The difference in stage of development at emergence, using the weighted mean K_D values for the entire runs, was 0.023 K_D-units (Table 3), which corresponds with about 6 days' growth at the prevailing temperatures. The 6-day difference in mean spawning dates corresponds with, approximately, a 12-day difference at time of emergence when the developmental rate was about

one-half that during the spawning period. Therefore, emergence of hatchery fry would be delayed by about 6 days. The actual delay was 5 days, i.e. 1 day short of the expected delay. The temperature records indicated a heat gain in the hatchery over the entire incubational period that would have advanced the mean emergence date by approximately 3 days. Only 1 day was unaccounted for following the earlier corrections and a net delay of 2 days over the entire incubational period is therefore indicated for the hatchery brood. However, where this quantity is small in relation to the length of the incubational period and, furthermore, is well within the accuracy of the method it can safely be concluded that no significant difference in developmental rates was observed between the creek and hatchery environments. This conclusion agrees with identical conclusions reached for the chum and pink salmon fry of the 1967 Hooknose Creek experiment, and the implication "that the combinations of water flows and fish densities as used in this [the Hooknose Creek] test were adequate in preventing density-related stressing factors to reach harmful levels in respect of the developmental rates" (Bams 1970) is fully supported by the results of the Tsolum River test.

The difference in mean stage of development at emergence of hatchery and creek fry differed between the two tests. By applying 99% confidence limits established for the difference between the total sample means (p. 0000) to the differences obtained in the Tsolum test and comparing with the corresponding Hooknose Creek test result, 0.047, a significant difference between the two tests is indicated. A similar calculation for the mean lengths data also shows a statistically significant reduction in the length differential (p. 0000; Table 3), which supports this conclusion. Evidently intergravel conditions in the hatchery at Headquarters Creek favored more advanced development by triggering migratory behavior at a later stage of development. The most likely reason for this difference was a greatly reduced amount of silt present in the intergravel interstices, although the higher loading density may also have been of importance (Bams unpublished data).

Efficiency of yolk conversion in the hatchery environment relative to that occurring in the creek is of paramount importance in evaluating the effectiveness of the incubation method. To compare growth rates and efficiency of yolk utilization between the two treatments fry lengths may be used as an index, provided the comparison is made at identical stages of development and average egg sizes were the same in both treatments (Bams 1970). The latter provision was satisfied by controlled allocation to both treatments of relatively large numbers of nonselected females throughout the adult run. Where the average stages of development, as expressed by the K_D-value, differed between the two fry runs at time of migration, the hatchery fry were converted to the stage of development of the creek fry by subtracting the difference in mean K_D-values and adding the equivalent growth increment (where $0.01\ K_D = 0.25$ mm). On the weighted means this conversion increased the length of the hatchery fry to 32.90 mm, i.e. 0.12 mm smaller than the corresponding mean of the creek fry, and on the unweighted sample means to 32.78 mm, i.e. 0.12 mm larger than the corresponding creek fry (Table 3). It follows that the overall growth rates in the two environments were virtually identical, and that yolk conversion efficiency in the hatchery environment was equal to that in the creek. The same conclusion was drawn from the Hooknose Creek data.

In both tests the hatchery method increased the numbers of fry per unit of available eggs. The present test showed higher survivals than were obtained in the Hooknose Creek experiment. Green egg to fry survival improved to 68.3% from 55% and survivals in the gravel boxes to 87.7% from 82%. The relatively low survivals at Hooknose were attributed to breakdowns in the water supply and intermittent, but sometimes prolonged, use of unfiltered water. Similar problems occurred at Headquarters with the earlier eggs prior to eyeing, as discussed earlier. Green egg to fry survival of these earlier eggs (Table 1) was 56.9%, which is in good agreement with the Hooknose data. The later Headquarters eggs, which were not exposed to such adverse conditions, boxes 3 and 4, reached levels of 78.0 and 79.7%, which constitutes a substantial improvement and clearly illustrates the importance of a reliable supply of continuously filtered water. Egg to fry survival in Headquarters Creek was 11.3%. This compares with a 14-year (1947–60) average survival of pink salmon in Hooknose Creek of 7.3% with a range of 0.9–37.2% (Parker 1962). Thus, there is no question about the greater efficiency of the total hatchery method in producing fry. Non-gravel incubation systems, including the previously mentioned Kleanza Creek pink salmon hatchery, commonly achieved even higher egg to fry survivals ($\geqslant$90%), but superior quantity was insufficient to offset inferior quality and the gains in numbers were not perpetrated to the returning adult stage.

The observed differential in survival in the gravel boxes between eggs that had been exposed to an earlier stressing period and those that had not, and indicated a "delayed mortality" of the unstressed eggs of about 8% (p. 1154) is of interest in this

connection. It can be postulated that the higher overall survivals as obtained in conventional hatcheries represent no significant gain in production as compared to our method, because this gain consists largely of inferior fry with an inherently low survival potential and hence would not contribute to increased survivals at the adult stage. Observations on brood raised in conventional baskets readily demonstrate the regular occurrence of live but noticeably underdeveloped and weak individuals that would appear unable to migrate from a gravel box.

Evaluation at the Adult Stage

The primary test criterion concerned the change in ratio of abundance of hatchery and creek progeny from time of release to time of adult return. The gain ratios were: at the fry stage, 6.04; at the adult stage, based on the actual mark returns, 5.97; based on the expanded results, marked fish, 6.27, and unmarked fish, 6.16. Evidently no appreciable change had occurred in the gain ratio achieved at the fry stage following release from the hatchery. According to the "criteria of success", as set out in the introduction, this single result, therefore, renders the test a "complete success". It was further demonstrated that no changes were introduced in the first generation of hatchery-produced adults that led to significant differences between the fish of the two treatments in respect of length, weight, fecundity, and times of passage through the fishery, stream entry, and egg deposition. It is concluded that under the conditions of this test the hatchery method achieved its primary goal of significantly increasing the survival rate from egg to returning adult stage, without introducing demonstrable adverse effects.

It would be premature to compare in detail the outcome of this single test with earlier hatchery efforts. Several operations must be completed before the general performance of the method will be known. Furthermore, North American efforts at raising pink salmon have been very limited in number and extent and seem poorly documented. Comparison with other propagation techniques, particularly spawning channels, will be essential, but is also better deferred until the performance of both the gravel-incubation method and the spawning channels are assessed further.

In general terms, this first test compares favorably with some of the best results reported for artificial propagation of pink salmon, e.g. some of the Russian pink salmon hatcheries. Kanid'yev et al. (1970) report "coefficients of downstream migration" (presumably survival, in percent of fry from potential egg deposition) for six "control rivers" and five hatcheries of Sakhalin for the years 1962–68 inclusive. The rivers showed 7-year averages of 15.6, 14.1, 16.5, 21.9, 7.6, and 12.8%, and the hatcheries 95.1, 96.7, 96.2, 94.3, and 94.3%. The unweighted mean gain ratio at the fry stage works out to 6.46. The paper claims a gain ratio of 7 to 10, in terms of adults required for production. At time of release hatchery fry are "usually larger or the same size and very rarely smaller" than naturally produced fry, due to a period of intensive feeding prior to release which, during the last decade, has been standard practice in Russian hatcheries. During "embryonic and larval development in hatcheries where abiotic conditions are close to natural conditions, young pink salmon differ hardly at all from natural pink salmon with respect to morphological characters..." Sea survival of the hatchery pink salmon is not known, because all hatcheries included in the report are on streams that also have natural production and, to my knowledge, no marking experiments have been carried out to differentiate between the two sources. Evidently it is inferred from the combined returns that there is little difference in sea survival between the two sources, but the paper does not present the necessary data.

General and Incidental Comments

The hypothesis advanced in the introduction concerning the nonselectivity of a significant part of normally occurring creek mortality in relation to subsequent survival to the adult stage is supported by the results. The hatchery brood was shown to be very similar to its naturally propagated counterpart in characteristics thought to reflect fry quality. The best support derives from the conclusion that the survival rates from fry to adult were equal in fish of both treatments. This evidence carries the important implication that any enhancement method that reduces creek mortality while retaining potential fry quality can result in similarly improved adult survivals. The upper limit of potential gains appears to be high: for the pink salmon employed in this test, best survival from green egg to fry was on the order of 80%. A seemingly unavoidable "delayed mortality" of eyed eggs of about 8% occurred following hatching, which may indicate that the very high survival figures obtained with some conventional hatchery methods (90–95%) are this high due to the inclusion of inferior stock that can survive to the pelagic fry stage in baskets or trays but not in a gravel medium.

A comment seems warranted regarding values obtained for sea survival and marking mortality. It was anticipated that sea survival prior to fishing mortality for unmarked and marked fish would be on the order of 3 and 1.5%, respectively. The final estimates of this experiment were for the unmarked

hatchery fish, 6.65%, Z = 2.71, and for the unmarked creek fish, 6.52%, Z = 2.73. Most reported estimates are considerably lower, e.g. Z = 3.15 for self-sustaining populations (Parker 1962), and Z = 2.95 for the Burke Channel–Bella Coola population (Parker 1965). Differential mortality introduced by marking was more seriously underestimated. Ratio of survival of unmarked over marked fish was anticipated at 2.0. The creek returns showed a ratio of 5.3, or, expressed as a ratio of instantaneous mortality rates, $Z_M/Z_U = 1.41$. Parker et al. (1963) reviewed five pink salmon marking experiments carried out between 1931 and 1948 and recorded values for S_U/S_M of 2.6–10.5, and for Z_M/Z_U of 1.31–1.57. In designing the experiment it was assumed that by extremely careful handling of the fish, the use of modern surgical equipment, and a supposedly safer anesthetic a reduction in marking mortality would be achieved. The results fell in the middle of the recorded range of values and, therefore, do not support this contention. Expected S-ratios for this type of marking experiment must still stand at about five.

Apparent selectivity of the fishery was noted in two instances. One concerned a pronounced selectivity for females over males. The sex ratio (male/female) in the catch was 0.73 for the hatchery fish and 0.70 for the creek fish and 1.87 and 1.46, respectively, in the escapement. Each of these ratios deviates significantly from unity, χ^2 from 8.91 to 17.85, $P < .01$. The discrepancy between catch and escapement on the combined sources is significant at $P = .000$, $\chi^2 = 43.00$. Jenkinson and Bilton (MS 1971) reported catch sample statistics for 1970 on mixed stocks and estimated the percentage males in area 12 at 47.3% and in area 13 at 45.0%. These figures compare to 42.0 and 41.1% for our hatchery and creek stock recoveries. Because the sex ratio was reversed in the escapement the conclusion is inevitable that the fishery was selective and that the ratios observed in the catch were not representative of the total stocks and, particularly, of the escapement. Because the fishery was exclusively by purse seine, selectivity is difficult to understand but its occurrence was documented. Following the same procedure used to estimate the total adult populations (using the sums of twice the known catch plus the escapement), the "true" sex ratios of the populations prior to fishing are estimated at 0.89 for the hatchery and 0.88 for the creek progeny.

The second discrepancy between catch and escapement concerned selectivity of the fishery for hatchery fish or, alternatively, for fish marked AdLV, the mark used to identify hatchery fish. This discrepancy is significant, $\chi^2 = 12.77$, $P < .001$. Inspection of Table 5 shows that the uneven distribution was consistent throughout the run and cannot be explained by differences in fishing pressure acting on a difference in time of passage through the fishing areas. This selectivity has one important consequence. It was estimated that a maximum of 50% of the marked fish caught in the fishery were recovered from the canneries. The catch recoveries favored the hatchery fish 370 to 310, which differs significantly from unity, $\chi^2 = 5.29$, $P < .05$. Because the main evaluation test was carried out on actual recoveries only, it follows that the test was biased in favor of the naturally propagated fish. Correction for this bias increases the overall survival rates for the hatchery brood to complete equality with the creek progeny, as was demonstrated in the text table on p. 1159.

A conspicuous irregularity in the fence entry records for the adult returns (Table 6, Fig. 5) warrants a comment. A difference between mean and median days of entry up to 2 days indicated a nonsymmetrical distribution of the runs in time. This event was attributed to a change in water flow in creek and river brought about by reducing the release of water from Wolf Lake, the main supply of water in the Tsolum system during the spawning season. Headquarters Creek flows before and after the change were approximately 26.5 and 18.5 ft^3/sec; the date of change was September 17. The cumulative entry records (Fig. 5) show an abrupt change in mean entry rates 2 days after the change in flow. This delay may reflect the time necessary for the fish to ascend the Tsolum River; the fish entering the weir the day after the change had already started their ascent and, therefore, still reflect the flow before the change. Of further interest is a difference between the sexes, shown by all three groups, in response to the flow change. The males responded immediately with a lower but constant rate for most of the remainder of the run, but the females showed first a decrease and then an increase in rate over several days before establishing the new rate that was maintained for the remainder of the run. These observations indicate a direct correlation between rate of flow and rate of migration during ascension of the river.

Further assessment of the same and additional test criteria remain to be done before the method should be considered proven. A first requirement is replication of the test in different years. Environmental conditions, and possibly biological characteristics of entire brood years, differ between years and may reflect in differential survival rates which could favor one or the other incubational treatment in different years. Another requirement is evaluation of change in variability of stock characteristics and survival potential associated with continuing ap-

plication of this method of artificial propagation. The method aims at retention of a broad genetic variability by mating the fish randomly in 5 × 5 groups and assignment of brood stock consistently and nonselectively throughout the parent run. We also appear to be dealing with only one small local stock (deme), and thus are not mixing different populations. Nevertheless, certain means of selection are likely to differ between the hatchery and creek treatments. For example, mate selection in nature may not be random (Hanson and Smith 1967), which could result in broader variability in size of offspring in the creek population. Distribution over spawning sites may not be random, in which case higher survivals in "prime areas" may favor certain types of fish in the natural environment that would not be so favored in the "all prime area" hatchery environment. As pointed out by Calaprice, many hatchery procedures are likely to affect the pattern of genetic variance and "the latter may be expected to result in a reduction of fitness in the populations undergoing selection" (Calaprice 1969). Continuing operations on successive generations may disclose trends of this nature.

Acknowledgments

Execution of the test was made possible by the competent technical assistance supplied by Messrs R. C. Wilson and D. G. Crabtree, and their contributions are gratefully acknowledged. I am indebted to Dr. R. R. Parker for assistance in developing the test design and for reviewing the manuscript. Several people of the Resource Development Branch of the Department of Fisheries and Forestry, Vancouver, B.C., supplied biological and technical information of importance to the project.

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Amine-Citrate Buffers for *p*H Control in Starch Gel Electrophoresis

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CLAYTON, J. W., AND D. N. TRETIAK. 1972. Amine-citrate buffers for *p*H control in starch gel electrophoresis. J. Fish. Res. Bd. Canada 29: 1169–1172.

Amine-citrate buffer systems for *p*H control in starch gel electrophoresis gave good resolution of some dehydrogenase isozymes. The pK's of three new amine buffers, N-(3-aminopropyl)-morpholine, pK_2 25 C, 6.12; N-(3-aminopropyl)-diethanolamine, pK_2 25 C, 6.90; and 1,3-bis(dimethylamino)-2-propanol, pK_2 25 C, 7.55, were determined at 5 C intervals in the range 10–40 C. These compounds, together with N, N-bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane (bis-Tris) and tris-(hydroxymethyl)-methylamine(Tris), provide a series of amine buffers with pK's at 0.5 unit intervals in the *p*H range 6.1–8.1.

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L'emploi de systèmes tampons amines-citrates pour le contrôle du *p*H dans l'électrophorèse sur gel d'amidon a fourni une bonne résolution de quelques isozymes de la déshydrogénase. Les valeurs de pK pour trois nouveaux tampons amines, N-(3-aminopropyl)-morpholine, pK_2 25 C, 6.12; N-(3-aminopropyl)-diéthanolamine, pK_2 25 C, 6.90 et 1,3-bis(diméthylamino)-2-propanol pK_2 25 C, 7.55 ont été déterminées à intervalles de 5 C sur une gamme de 10 à 40 C. Ces composés, de même que N, N-bis(2-hydroxyéthyl)-iminotris(hydroxyméthyle)-méthane (bis-Tris) et tris-(hydroxyméthyl)-méthylamine(Tris), fournissent une série de tampons amines avec des valeurs de pK à intervalles de 0.5 unités sur la gamme de *p*H de 6.1 à 8.1.

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STARCH gel electrophoresis of specific proteins and enzymes has become one of the central techniques of biochemical genetics. One of the advantages of the technique is that the investigator may, in principle, vary the *p*H of the gel medium over a range sufficient to maximize the differences in mobility of the particular proteins under study. In practice, some limitations arise that are related to the necessity of providing adequate *p*H buffering capacity while at the same time keeping the ionic strength of the gel as low as possible in order to minimize electrical currents and undesirable heating of the gel. In studies of enzymes it is also essential to use a buffer system that is compatible with the preservation of enzymatic activity.

Our search for buffer systems that might be useful for starch gel electrophoresis, particularly for the resolution of the abundant isozymes of dehydrogenases in fish, has been influenced by earlier investigations of dehydrogenases that showed the protective effects of coenzymes, citrate ions, and polyhydroxy compounds. Citrate and coenzymes protected lactate dehydrogenase from inactivation by urea (Di Sabato and Kaplan 1965) and also promoted the reactivation of malate dehydrogenase following inactivation by guanidine hydrochloride (Chilson et al. 1965a). More extensive investigations have shown the beneficial effect of citrate in the reactivation of lactate and malate dehydrogenase after guanidine hydrochloride inactivation, as well as in the protection of malate dehydrogenase from heat inactivation (Chilson et al. 1966). In studies of the freezing and thawing of several enzymes it was found that some coenzymes and a number of polyhydroxy compounds prevented the loss of catalytic activity and also, evidently, inhibited the dissociation of multimeric enzymes into their monomeric subunits (Chilson et al. 1965). A general conclusion that may be drawn from these studies is that citrate, coenzymes, and, to some extent, polyhydroxy compounds probably exert a protective effect on the catalytic activity of several dehydrogenases. It therefore appeared likely that buffers for electrophoresis of these enzymes could advantageously be made up with citric acid buffered with an appropriate polyhydroxyamine. The combination of tris-(hydroxymethyl)-methylamine (Tris) buffered citrate has

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proven to be useful in a number of investigations (Clayton and Gee 1969; Clayton et al. 1971; Williscroft and Tsuyuki 1970).

It has become clear that it would be desirable to have a series of amines available to buffer citrate over a range of *p*H in order to bring out all the variability present in some isozyme systems. This paper describes the ionization constants and the suitability for electrophoretic studies of a number of amine buffers that range in pK from 6.1 to 8.1 in approximately 0.5 unit intervals.

Materials and Methods

The whitefish (*Coregonus clupeaformis*) used in this investigation were collected from Clear Lake, Riding Mountain National Park, Man. The walleye (*Stizostedion vitreum vitreum*) was taken from Lake of the Woods, Ont.

The amine buffers were obtained from the following suppliers: N, N-bis (2-hydroxyethyl) iminiotris (hydroxymethyl) methane (bis-Tris), General Biochemicals, Chagrin Falls, Ohio; 2-amino-2-methyl-1, 3-propanediol, Sigma Chemical Co., St. Louis, Mo.; N-(3-aminopropyl)-morpholine, N-(3-aminopropyl)-diethanolamine, and 1,3-bis (dimethylamino)-2-propanol, Aldrich Chemical Co., Milwaukee, Wisc.; tris-(hydroxymethyl)-methylamine, "Analar grade," B.D.H. Chemicals, Toronto, Ont.; triethanolamine was "Baker Analyzed" from Canlab, Toronto, Ont. These amines were used for titrations and also as *p*H buffers in electrophoresis as received from the suppliers. Other chemicals used were of laboratory reagent grade.

Starch gel electrophoresis was conducted with the apparatus described by Tsuyuki et al. (1966). The general methods of extract preparation, electrophoresis, and enzyme staining have been described previously (Clayton and Gee 1969; Clayton et al. 1971). As in the previous work, starch was gelled in buffers prepared by adjusting 0.00200 M citric acid to the desired *p*H with an appropriate amine at room temperature, approximately 22 C. The electrode buffers were made similarly with 0.0400 M citric acid. These concentrations give ionic strengths similar to the citric acid/phosphate buffers described by Fine et al. (1963).

In the present work, muscle extracts were prepared by homogenizing tissue with a distilled water solution of nicotinamide adenine dinucleotide (NAD), 300 mg/liter in a ratio of 1 g muscle to 3 ml NAD solution.

The pK values for the amines were determined graphically from titration curves obtained with an automatic titrator. The titration equipment was obtained from Radiometer, Copenhagen, and consisted of a model 26 *p*H meter, a model GK2021c combination calomel/glass electrode, a SBR 2c Titrigraph and a SBUld Syringe Burette. The meter and electrode were standardized with buffers recommended by Bates (1964).

The amines (8 ml, 0.050 M solution) were titrated with 0.200 N hydrochloric acid under nitrogen in a jacketed, temperature-controlled vessel. The actual temperature of the solution titrated was observed directly with a small thermometer. For amines with two nitrogen atoms per mole the titration of the more acidic nitrogen was conducted on a stock solution that was already partly neutralized with hydrochloric acid. Eight titrations were made with each amine at each temperature.

Buffer values $\frac{\Delta B}{\Delta pH}$ (Van Slyke 1922; Bates 1964) were determined by measurement of the change in *p*H produced by the addition of 1 ml of standardized hydrochloric acid or sodium hydroxide to 100 ml of amine-citrate buffer. The concentration of acid or base used was determined in a trial experiment to be sufficient to produce a *p*H change of 0.1–0.2 unit. The temperature of the amine-citrate buffer was controlled at 25± 0.1 C and each buffer was tested in triplicate.

The conductivities at 25 ± 0.1 C of amine-citrate buffers were measured in the usual way with a conductivity "bridge" and platinized cell.

Results and Discussion

The application of amine-buffered citric acid to starch gel electrophoresis is shown in Figs. 1–3. Malate dehydrogenase isozymes in white muscle of a C^1C^1 homozygous walleye (Clayton et al. 1971) are well separated at *p*H's between 6.1 and 7.5 (Fig. 1), but in the much-used Tris-citrate at *p*H 8.0 two of the isozymes are no longer clearly separable. Lactate dehydrogenase in red muscle of a FF homozygous lake whitefish (Clayton and Franzin 1970) appears as two groups of isozymes in the *p*H range 6.1–8.5 (Fig. 2). However, the existence of multiple muscle-type isozymes, the least anodal group, is only evident at *p*H 7.8, 8.0, and 8.5, while the multiplicity of the heart-type isozymes is clearly evident at all *p*H's studied. The multiplicity of both sets of isozymes that has been used as evidence for tetraploidy in salmonids (Klose et al. 1968; Ohno et al. 1968; Massaro and Markert 1968) would be completely missed in this species if electrophoresis was carried out at *p*H 7.5 or lower. Electropherograms of lake whitefish malate dehydrogenase (MDH) show (Fig. 3) a fish with an apparent symmetrical heterozygous phenotype similar to that found in rainbow trout and Pacific salmon by Bailey et al. (1970). The full multiplicity of MDH isozymes, with the most anodal isozymes occurring as progressive sets of one, two, and three isozymes each, was seen only at *p*H 6.5 and 6.1. At *p*H 8.0, the isozyme pattern is so contracted that it no longer has any resemblance to the *p*H 6.1 pattern or to the phenotypes shown by Bailey et al. (1970). It was suggested in the introduction that amines with hydroxyl groups would be desirable in order to combine the protective effects of polyhydroxy compounds with the buffering capacity of amines. In our search for

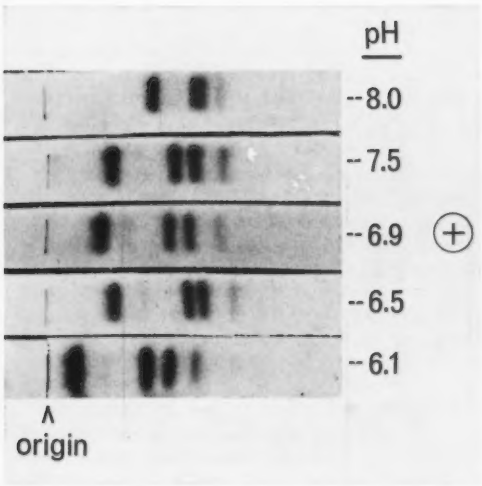

FIG. 1. Walleye malate dehydrogenase electrophoresis amine-citrate buffers: *p*H 6.1, N-(3-aminopropyl)-morpholine; *p*H 6.5, bis-Tris; *p*H 6.9, N-(3-aminopropyl)-diethanolamine; *p*H 7.5, 1,3-bis(dimethylamino)-2-propanol; *p*H 8.0, Tris.

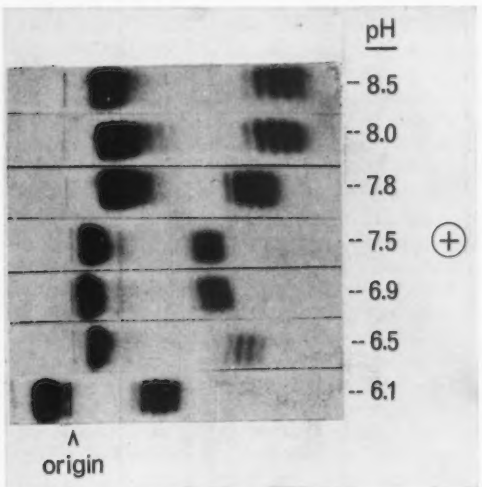

FIG. 2. Lake whitefish lactate dehydrogenase electrophoresis, amine-citrate buffers; *p*H 6.1, N-(3-aminopropyl)-morpholine; *p*H 6.5, bis-Tris; *p*H 6.9, N-(3-aminopropyl)-diethanolamine; *p*H 7.5, 1,3-bis(dimethylamino)-2-propanol; *p*H 7.8, triethanolamine; *p*H 8.0, Tris; *p*H 8.5, 2-amino-2-methyl-1,3-propanediol.

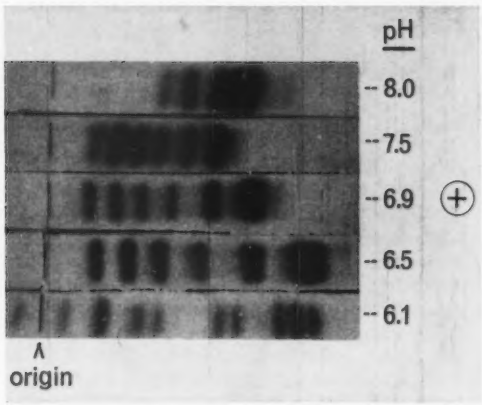

FIG. 3. Lake whitefish malate dehydrogenase electrophoresis amine-citrate buffers: *p*H 6.1, N-(3-aminopropyl)-morpholine; *p*H 6.5, bis-Tris; *p*H 6.9, N-(3-aminopropyl)-diethanolamine; *p*H 7.5, 1,3-bis(dimethylamino)-2-propanol; *p*H 8.0, Tris.

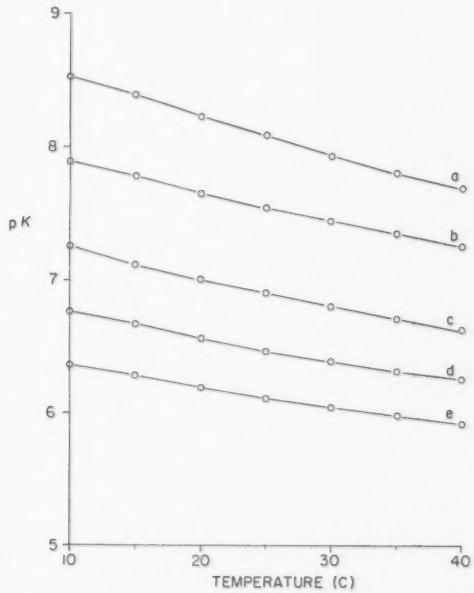

FIG. 4. Effect of temperature on pK of amine buffers: a, Tris; b, 1,3-bis(dimethylamino)-2-propanol (second ionization); c, N-(3-aminopropyl)-diethanolamine (second ionization); d, bis-Tris; e, N-(3-aminopropyl)-morpholine (second ionization).

TABLE 1. pk apparent for amines in 0.05 M solution.

Temp.	N-(3-aminopropyl)-morpholine $H_2NCH_2CH_2CH_2N\langle O \rangle$ pK_2	Bis-Tris $(HOCH_2CH_2)_2NC(CH_2OH)_3$ pK	N-(3-aminopropyl)-diethanolamine $H_2NCH_2CH_2CH_2N(CH_2CH_2OH)_2$ pK_2	1,3-bis(dimethylamino)-2-propanol $(CH_3)_2NCH_2CHOHCH_2N(CH_3)_2$ pK_2	Tris $H_2NC(CH_2OH)_3$ pK	Tris $H_2NC(CH_2OH)_3$ $-\log K_{bb}$*
40±0.2 C	5.91±0.013	6.25±0.012	6.63±0.019	7.25±0.010	7.69±0.014	7.6774±0.0011
35±0.1 C	5.98±0.008	6.32±0.016	6.71±0.019	7.35±0.014	7.81±0.018	7.8031±0.0010
30±0.1 C	6.04±0.017	6.39±0.016	6.80±0.009	7.44±0.017	7.93±0.019	7.9344±0.0010
25±0.2 C	6.12±0.011	6.47±0.013	6.90±0.015	7.55±0.006	8.09±0.015	8.0746±0.0006
20±0.2 C	6.19±0.012	6.56±0.015	7.00±0.015	7.64±0.015	8.23±0.018	8.2138±0.0009
15±0.2 C	6.28±0.019	6.67±0.029	7.11±0.027	7.78±0.020	8.39±0.019	8.3616±0.0008
10±0.3 C	6.36±0.028	6.76±0.026	7.25±0.031	7.89±0.039	8.53±0.017	8.5164±0.0008
	pK_1		pK_1	pK_1		
25±0.2 C	9.99±0.015		10.26±0.013	9.37±0.015		

*Data of Bates and Hetzer (1961).

TABLE 2. Buffer value and conductance of 0.00200 M amine-citrate buffers at 25 C.

Amine buffer	Nominal *p*H	Buffer value × 10^3 HCl $\left(\frac{\Delta B}{\Delta pH}\right)$	Buffer value × 10^3 NaOH	Specific conductance × 10^4 (*mhos/cm*)
N-(3-aminopropyl)-morpholine	6.1	2.9	2.8	3.6
Bis-Tris	6.1	3.7	4.2	3.7
Bis-Tris	6.5	6.2	6.7	4.2
Bis-Tris	6.9	10.0	9.1	4.6
N-(3-aminopropyl)-diethanolamine	6.9	2.4	2.5	4.0
1,3-bis(dimethylamino)-2-propanol	7.5	2.1	2.5	4.2
Triethanolamine	7.5	3.7	4.2	5.2
Tris	7.5	2.0	2.5	5.0
Tris	8.0	4.6	5.3	5.1

amines with pK's in the *p*H range 6–8, we were not always able to find compounds that combined both properties of desirable pK and the presence of a large number of hydroxyl groups. The amines we have investigated do seem to perform well in our electrophoretic procedures (Figs. 1–3), in spite of the number of hydroxyl groups.

A solution of coenzyme (NAD) was used to make the tissue extracts. We have found that the level of enzymatic activity is usually enhanced in extracts with NAD compared to water extracts. We have also found that much clearer electropherograms are obtained, with some enzymes, when coenzyme is also added to the starch and electrode buffers (Clayton et al. unpublished data). In the present work this further addition of coenzyme did not seem to be necessary.

The results of the pK determinations (Table 1, Fig. 4) show that a set of amine buffers is now available with intervals of pK of about $\frac{1}{2}$ unit from *p*H 6 to 8. The new or little-used compounds, N-(3-aminopropyl)-morpholine, N-(3-aminopropyl)-diethanolamine, and 1, 3-bis (dimethylamino)-2-propanol, have pK's that are temperature-dependent like many other amines (Bates 1961). We have measured the first dissociation for these three compounds only at 25 C. These ionizations are probably too alkaline to be of any particular interest as biological *p*H buffers. In the original description of bis-Tris (Lewis 1966), the pK at 25 C was reported to be 6.46; the present value of 6.47 is in good agreement. The variation of pK with temperature for bis-Tris has apparently not previously been reported. The present results confirm the prediction of Lewis (1966) that the pK of bis-Tris would vary with temperature in a manner similar to other amines.

Our data for the pK apparent of Tris may also be compared (Table 1) with pK_{bh} determinations of Bates and Hetzer (1961). The pK_{bh} values contain numerous corrections in comparison with the present results; however, it may be seen that the agreement with the present data is sufficient for all practical purposes related to the choice of a *p*H buffer. We have not investigated the effect of concentration on the pK's of these amines because it has already been shown by Bates (1961, 1964) that the pK's of amines are little affected by concentration at near neutral *p*H of biological interest.

In addition to the amines listed in Table 1, we have found triethanolamine and 2-amino-2-methyl-1, 3-propanediol to be satisfactory as *p*H buffers in electrophoresis (Fig. 2). The physical properties of these amines and many others have been reported by Bates (1961).

The term "buffer value" was suggested by Van Slyke (1922) to describe the effectiveness of a *p*H buffer in resisting *p*H change resulting from the addition of acid or base. Buffer value was defined quantitatively as $\frac{dB}{dpH}$, the slope of the titration curve at any particular *p*H. In practice it is easier to use $\frac{\Delta B}{\Delta pH}$ where the ΔB is a macroscopic addition of base (or acid) and ΔpH is the macroscopic *p*H change. We have measured (Table 2) buffer values and conductivities for a number of amine-citrate buffer systems of the concentration normally used as the gel buffer in starch gel electrophoresis. The results show the superiority of bis-Tris as a *p*H buffer over the other amines. This is due mainly to the way in which these buffers are prepared, whereby the amine is added in quantity sufficient to titrate the citric acid, and thus the concentration of monofunctional amines like bis-Tris is considerably higher than that of difunctional amines like N-(3-aminopropyl)-morpholine. The reduced buffer capacity of some of the difunctional amine buffer systems does not seem to have had any deleterious effect on the isozyme resolution that actually is obtained in electrophoresis (Figs. 1–3). There were no important differences in the conductivity of the various buffer systems (Table 2). The general trend for lower conductivities at lower *p*H is convenient to a practical electrophoretic procedure, since it is usually necessary to increase the voltage to compensate for decreased cationic character of proteins as the *p*H decreases.

In summary, this investigation illustrates the good resolution of the multiple isozymes of some fish dehydrogenases that is produced in starch gel electrophoresis when the gel *p*H is controlled by amine-citrate buffer systems. These systems produce electrophoretic separations that may be interpreted directly in terms of the *p*H of the medium and are not influenced by the character of any particular buffer species. Three previously unused amines together with Tris and bis-Tris provide a series of amine buffers with pK's at 0.5 *p*H unit intervals in the *p*H range 6.1–8.1. The observed differences in the buffering capacity of the electrophoresis buffers did not seem to affect the quality of the electropherograms. The ionization of all the amines tested was, as expected, markedly temperature-dependent, and reproducible electropherograms are likely to depend upon adequate temperature control during both buffer preparation and electrophoresis.

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Morphological and Myogen Comparison of Johnny and Tessellated Darters and Their Hybrids, Genus *Etheostoma*, Near Ottawa, Canada

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MCALLISTER, D. E., P. JOLICOEUR, AND H. TSUYUKI. 1972. Morphological and myogen comparison of johnny and tessellated darters and their hybrids, genus *Etheostoma*, near Ottawa, Canada. J. Fish. Res. Bd. Canada 29: 1173–1180.

Ten morphological characters and two electrophoretic characters were studied in nearly 200 specimens of johnny and tessellated darters from the Ottawa area. The data were analyzed using a simple character index and principal component analysis. The frequency distribution of the character index was trimodal, and specimens could be resolved into three groups: those typical of johnny and tessellated darters, and a group of intermediates. The two extreme groups were interpreted as representing two valid species, *Etheostoma nigrum* Rafinesque and *Etheostoma olmstedi* Storer, and part of the intermediate group was inferred to consist of interspecific hybrids. Hybrids were estimated to constitute less than 7.6%. There was no clear evidence of backcrossing. Most specimens could be clearly identified with one or the other of the two species. A key is provided.

MCALLISTER, D. E., P. JOLICOEUR, AND H. TSUYUKI. 1972. Morphological and myogen comparison of johnny and tessellated darters and their hybrids, genus *Etheostoma*, near Ottawa, Canada. J. Fish. Res. Bd. Canada 29: 1173–1180.

Nous avons étudié dix caractères morphologiques et deux caractères électrophorétiques d'environ 200 spécimens raseux-de-terre et de dards tesselés provenant de la région d'Ottawa. Nous avons analysé les données à l'aide d'une combinaison (indice) simple des caractères morphologiques et par la méthode des composantes principales. La distribution de fréquence de la combinaison de caractères est trimodale, et nous avons pu distinguer trois groupes de spécimens: ceux qui sont typiques du raseux-de-terre, ceux qui sont typiques du dard tesselé, ainsi qu'un groupe d'intermédiaires. Nous croyons que les spécimens des deux premiers groupes appartiennent à deux espèces valides, *Etheostoma nigrum* Rafinesque et *Etheostoma olmstedi* Storer, et nous concluons qu'une partie des spécimens intermédiaires sont des hybrides interspécifiques forment moins de 7.6% du total. Les données ne fournissent pas d'indications claires de rétrocroisement. La majorité des spécimens peuvent être identifiés comme appartenant à l'une ou l'autre des deux espèces, et nous donnons une clef.

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THE taxonomic position accorded the tessellated darter, *Etheostoma olmstedi* Storer, 1842 relative to the johnny darter, *Etheostoma nigrum* Rafinesque, 1820, has varied. Stone (1947) compared the two forms inside and outside one area of sympatry using discriminant function analysis and suggested the two were distinct at the species level, but reserved final decision. Hubbs and Lagler (1964) treated *E. olmstedi* as a subspecies of *E. nigrum*. Cole (1958, 1965, 1967, 1972), in detailed studies of the subgenus *Boleosoma*, concluded that the two were valid species and in the latter study subdivided *E. olmstedi* into four subspecies and *E. nigrum* into perhaps three subspecies. Zorach (1971) cast doubt on Cole's

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recognition of *E. olmstedi atromaculatum* as distinct from *E. o. olmstedi*. Little Canadian material and none from west of Lac des Deux Montagnes in the Ottawa River was included in Cole's studies. Consequently subspecific designations will not be employed in the present paper. Aside from brief mentions in Cole (1958, 1972), which mention a hybrid swarm, there has been no published reports of hybrids between the two darters.

Etheostoma nigrum and *E. olmstedi* are sympatric in the Ottawa area (McAllister 1968). The area thus offers the opportunity for studying the border between species and subspecies in terms of the distinctiveness of these two forms. The present study considers the taxonomic status of the two forms using morphological and electrophoretic data analyzed by a simple character index and principal component analysis. For convenience and to avoid prejudice, the two taxa will initially be referred to simply as *nigrum* and *olmstedi* and the group of specimens with characteristics between the two taxa, as intermediates.

Materials and Methods

Thirty-four collections comprising 132 specimens from 25 stations in the Ottawa River and its tributaries in Ontario and Quebec within 40 miles of Ottawa, were studied morphologically. These will be referred to as the first sample, around which most of the discussion revolves. The collections were randomly taken except that 15 of the specimens in four collections from stations with intermediates were included so that areas of contact of the two forms could be studied in more detail. Ten morphological characters were then studied on each of the 132 specimens. Two myogen characters were studied in 21 of these specimens by starch gel electrophoresis employing methods described previously (Tsuyuki et al. 1968). These characters are listed below as X_1–X_{12}. Morphological and electrophoretic data for individual specimens have been deposited in the Ichthyology Unit, National Museum of Natural Sciences.

A second lot of 64 specimens, called the second sample, from the Ottawa area was also examined by starch gel electrophoresis. Full morphological data were not recorded for these specimens.

The specimens for electrophoretic studies were shipped fresh frozen with dry ice to the Vancouver Laboratory of the Fisheries Research Board where only white skeletal muscles were used in the preparation of protein extracts. The electrophoregrams revealed two zones labelled A and B, usually present singly in a specimen, but sometimes together and labelled AB. The protein molecules involved in zones A and B are somewhat similar to the allelic variants found in the sablefish, *Anoplopoma fimbria* (Tsuyuki and Roberts 1969), and may involve protein molecules consisting of only single polypeptides.

Ten morphological characters (see list below) were studied in the first sample of 132 specimens which had been roughly allotted to species. Character states or counts typical of *olmstedi* were given a value of one, typical of *nigrum* a value of zero, and those frequently encountered in both, a value of 0.5. The values for all of the characters were then totalled for each specimen, yielding an unweighted simple character index ranging from zero to 10. Specimens with indices of 0–3.5 were then reclassed as *nigrum*, 4–6 as intermediates, and 6–10 as *olmstedi*; these are referred to as the preliminary identifications. Of these, 54(41%) were *nigrum*, 18 (14%) intermediates, and 60 (46%) *olmstedi*.

Similarly a simple character index based on fewer characters was constructed for the second sample of specimens. Here, 36 (56%) can be classed as *nigrum*, 8 (12%) as intermediates, and 20 (31%) as *olmstedi*.

The results yielded by the simple character index on the first sample are very similar to those obtained by the statistical procedures discussed below, but the latter are preferred because their calculation does not require arbitrary decisions on the part of the taxonomist and extracts more fully the numerical information present in the data.

Although the technique of discriminant function analysis has been used successfully in several taxonomic problems, its use here would have required pure reference samples of *E. olmstedi* and *E. nigrum*. Since the present data presumably consisted of a mixed collection of specimens of the two species, plus possibly some intermediate specimens, principal component analysis was tried as a substitute for discriminant analysis. Calculations were carried out on a Hewlett-Packard digital computer. In principle, principal components are appropriate for the analysis of a sample from a single homogeneous multivariate population; in practice, however, when principal component analysis is applied to an heterogeneous sample made up of specimens from two or several populations, heterogeneity may be expected to loom large relative to variation within single populations, and differences between populations should come out on the principal components having the largest variances. Examination of results (for instance by means of scatter diagrams or histograms) is necessary in order to see which principal components bring out differences between populations.

The characters used in the construction of the simple character index and in the principal components analysis are listed below. The values awarded the different character states of nonmeristic characters in the principal components analysis are indicated after each character state. For numeric characters the actual counts were used.

X_1 : Upper lip not extending beyond snout, $X_1 = 1$
Upper lip extending beyond snout, $X_1 = 2$
X_2 : Nape not scaled, $X_2 = 1$
Nape partially scaled, $X_2 = 2$
Nape completely scaled, $X_2 = 3$
X_3 : Cheek not scaled, $X_3 = 1$
Cheek partially scaled, $X_3 = 2$
Cheek completely scaled, $X_3 = 3$
X_4 : Breasts not scaled, $X_4 = 1$
Breast partially scaled, $X_4 = 2$
Breast completely scaled, $X_4 = 3$
X_5 : Number of complete dark bars across caudal fin; what was counted was the number of dark vertical

bars extending most of the way across the caudal fin

X_6 : Number of soft dorsal rays (the last two being counted as one)

X_7 : Number of pectoral rays

X_8 : The number of scales above the lateral line was counted downwards and posteriorly from behind the first dorsal spine and did not include the lateral line scale

X_9 : Infraorbital canal not complete, $X_9 = 1$
Infraorbital canal complete, $X_9 = 2$

X_{10}: Number of preoperculomandibular pores

X_{11}: Electrophoretic band "A" absent
Electrophoretic band "A" present

X_{12}: Electrophoretic band "B" absent
Electrophoretic band "B" present

X_{11} and X_{12} were not used in calculating the character indices.

Results

Table 1 compares the frequencies of the character states of the 12 characters in the first sample of *nigrum*, the intermediates, and *olmstedi* following the preliminary identifications. The two species tended to have different character states with the intermediates falling in between.

Certain characters showed greater discriminatory value between the two species. This was measured by finding the line of best separation and determining the percentage correctly identified of the two species on either side of the line in relation to the total number of specimens of the two species (neglecting the intermediates). The characters can be ranked in order of discriminatory value from high to low as follows: scalation of breast 96%, number of caudal bars 92%, myogen band B 92%, number of soft dorsal rays 90% (94% in Cole 1965), scalation of cheek 89%, scalation of nape 87%, myogen band A 85%, lip position relative to snout 79%, number of pectoral rays 77% (78% in Cole 1965), number of preoperculomandibular pores 77%, number of scales above lateral line 69%, completeness of infraorbital canal 62%. The probabilities that these differences are due to chance, as measured by chi-square tests, is shown in Table 1. For most characters the probabilities are less than .001.

The 92% discriminating power of the B myogen band, and the 85% of the A myogen band is based on only about a dozen specimens of the first lot. If we examine myogen band data for the second sample of 64 specimens (less well studied morphologically) we find that the discriminating power of the A band is about 65% and the B band is about 67%. Results for the first sample are probably partly due to chance in the small sample. Results for the second sample are probably more representative, although not wholly satisfactory in that the specimens cannot be allotted to groups with the same certainty as the first sample; their real distinguishing power would probably be somewhat higher.

Figure 1 shows good agreement between preliminary identifications of specimens (as *nigrum*, *olmstedi*, and intermediates) based on the simple character index and the numerical value of the character index yielded by the first principal component of the correlation matrix of the 10 morphological characters. Some statistics are given in Table 2. It is noteworthy that the first principal component behaves as a satisfactory continuous variate even though it is made up of meristic and qualitative characters. Only the first principal component was found to possess this desirable property and to bring out variation of taxonomic interest. The numerical value of the character index based on the first principal component was calculated, in the case of each individual specimen, from the formula

$$Y = \sum_{i=1}^{10} (\mu_{1i}/s_i)\ (X_i - \bar{X}_i),$$

the weights (μ_{1i}/s_i) being those given in Table 2.

Testing for the Presence of Natural Hybrids

Examination of the frequency histogram of the first principal component (Fig. 1) suggests that intermediate specimens may be unexpectedly abundant and form a third minor peak between the two major peaks which correspond to *olmstedi* and *nigrum*. One difficulty in the analysis of this collection is that hybrid specimens are not known a priori to be present. On the other hand, in seeking a test for the possible presence of hybrids, a statistical procedure making use of preliminary identifications (as "intermediates", etc.) would be open to the criticism that the taxonomist's judgments are based on the same data as the character index. For these reasons, special efforts were made to find a statistical approach in which the collection is considered initially as a homogeneous whole and then hypothesized to be made up of specimens from the smallest acceptable number of statistical populations.

The hypothesis that all specimens belong to a single normal population is obviously untenable, as shown by the marked bimodality of the frequency histogram of the character index (Fig. 1); this graphical indication can also be confirmed by a kurtosis test based on sample cumulants (Table 3). Next, we consider the hypothesis that the collection is a mixture of specimens from two normal popu-

TABLE 1. Comparison of characters in *E. nigrum*, intermediates, and *E. olmstedi*.

	X_1 Upper lip		X_2 Scalation[a] (Nape)			X_3 Scalation[a] (Cheek)			X_4 Scalation[a] (Breast)			X_8 No. scales above lateral line				X_5 No. caudal bars									
	Beyond Snout	Even Snout	++	+	0	++	+	0	++	+	0	3	4	5	6	2	3	4	5	6	7	8	9	10	11
nigrum n = 55	4	50	2	13	39	0	11	43	0	5	49	20	32	2	0	1	19	29	4	1	0	0	0	0	0
intermediates n = 18	5	13	4	13	1	5	10	3	2	11	5	1	14	3	0	0	0	6	6	4	1	1	0	0	0
olmstedi n = 60	40	20	41	19	0	37	22	1	33	27	0	1	34	24	1	0	0	4	12	14	14	9	4	2	1
% separation (neglecting intermediates)	$\frac{90}{114} = 79\%$ $P = <.001$		$\frac{99}{114} = 87\%$ $P = <.001$			$\frac{102}{114} = 89\%$ $P = <.001$			$\frac{109}{114} = 96\%$ $P = <.001$			$\frac{79}{114} = 69\%$ $P = <.001$				$\frac{105}{114} = 92\%$ $P = <.00$									

	X_6 No. soft dorsal rays							X_7 No. pectoral rays					X_9 Infraorbital canal		X_{10} No. preoperculo-mandibular pores								X_{11} Band A		X_{12} Band B	
	10	11	12	13	14	15	16	10	11	12	13	14	I[b]	C[b]	5	6	7	8	9	10	11	12	P[c]	A[c]	P[c]	A[c]
nigrum	5	22	22	5	0	0	0	0	16	33	4	1	54	0	1	6	2	13	18	13	1	0	8	0	0	8
intermediates	1	3	8	3	2	1	0	0	1	9	8	0	17	1	0	0	2	0	9	6	1	0	8	0	7	1
olmstedi	0	0	6	22	28	3	1	0	1	20	37	2	43	17	0	0	1	0	11	25	22	1	2	3	4	1
% separation (neglecting intermediates)	$\frac{103}{114} = 90\%$ $P = <.001$							$\frac{88}{114} = 77\%$ $P = <.001$					$\frac{71}{114} = 62\%$ $P = <.001$		$\frac{88}{114} = 77\%$ $P = <.001$								$\frac{11}{13} = 85\%$ $P = >.05$		$\frac{12}{13} = 92\%$ $P = <.02$	

[a] ++ = completely scaled, + partly scaled, 0 scales absent.
[b] I = incomplete, C = complete.
[c] P = present, A = absent.

lations with the same dispersion, and estimate the parameters of the mixture (Table 4) following the procedure discussed by Rao (1952, section 8b.6). For this hypothesis to be acceptable, expected frequencies should match observed frequencies satisfactorily. However, the test of goodness-of-fit yields a significant chi-squared ($\chi^2 = 20.346$ with 7 df; $P < .01$), and examination of Table 5 and Fig. 2 shows that the lack of fit is due mostly to the unexpected abundance of intermediate specimens in central classes. Consequently the third central peak seems too pronounced to be attributed purely to random, and it may correspond to the presence of hybrids in the collection. As a corollary, the estimates given in Table 4 cannot be considered as valid. Unfortunately, there is no formal procedure at present for fitting a mixture of three normal populations, but examination of Table 5 and Fig. 3 suggests that such a trimodal distribution would fit the data satisfactorily. There is no clear evidence of backcrossing between hybrids and the parental forms although the small peaks between the three peaks might be due to backcrossing. Certainly backcrossing is not extensive nor is there swamping. In those cases the hybrid peak would be less prominent and the depth of the trough between the parental peaks would be considerably reduced. The excess of the observed frequency (18) over the expected frequency (7.94) of intermediate specimens may perhaps be used as an estimate of the number of hybrids present in the collections. This yields an estimate of approximately 10 hybrids on 132 specimens or 7.6% of the sample, a slight overestimate because relatively more samples were taken in the area of contact. Our interpretation of part of the intermediate specimens as hybrids is consistant with Hubb's report (1955) that the vast majority of interspecific hybrids possess character indices whose values are intermediate between those of their parental forms.

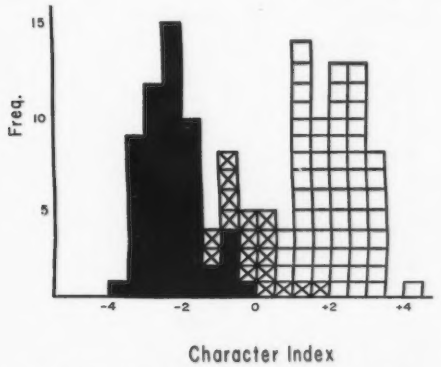

FIG. 1: Relation between the numerical value of the principal component character index and preliminary identification: ■ = *Etheostoma nigrum*, □ = *E. olmstedi*, ⊠ = intermediate specimen.

TABLE 2. Means $\bar{X}_i$, standard deviations s_i, direction cosines u_{1i} of major axis of correlation matrix and coefficients u_{1i}/s_i of character index based on the first principal component for the morphological characters as coded.

Character	$\bar{X}_i$	s_i	u_{1i}	u_{1i}/s_i
1	1.371	.4850	.2430	.5011
2	2.038	.8140	.3988	.4899
3	1.962	.8234	.3975	.4828
4	1.826	.7863	.4013	.5103
5	5.212	1.7992	.3380	.1878
6	12.523	1.2809	.3410	.2662
7	12.280	.7238	.2549	.3522
8	4.076	.6494	.2522	.3883
9	1.121	.3276	.1762	.5379
10	9.386	1.3402	.2697	.2013

TABLE 3. Sums of powers of deviations and sample cumulants ("K-statistics") for mixed sample of 132 specimens.

	1st	2nd	3rd	4th
Sums of powers	0	646.57	-97.24	4876.9
Sample cumulants	0	4.9357	-.7537	-35.814

TABLE 4. Estimates of the parameters of the hypothetical mixture of two normal populations.

Means:	$\mu_1 = -2.1498$	$\mu_2 = 1.9721$
Common standard deviation:	$\sigma = 0.83433$	
Proportion of mixture:		
	47.84% from the first population	

Myogen bands offered an independent method of checking the preliminary identifications of the first lot of specimens made on a morphological basis. As can be seen in Fig. 3 all but two of the 13 specimens identified as one or the other of the parental forms had the single appropriate band. All but one of the eight specimens identified as intermediate had both bands (Fig. 4). The additional characters confirm the preliminary identifications and indicate that the simple character index (and by association the

TABLE 5. Test of goodness-of-fit of the hypothesis that the distribution of the character index based on the first principal component, Y, is made up of two normal distributions.

Class	Expected absolute frequency		Observed absolute frequency		Chi-squared term
$Y < -5$	.02		0		
$-5 < Y < -4.5$	.13		0		
$-4.5 < Y < -4$	.68	9.71	0	10	.009
$-4 < Y < -3.5$	2.49		1		
$-3.5 < Y < -3$	6.39		9		
$-3 < Y < -2.5$	11.58		12		.015
$-2.5 < Y < -2$	14.79		15		.003
$-2 < Y < -1.5$	13.32		10		.828
$-1.5 < Y < -1$	8.47		4		2.359
$-1 < Y < -0.5$	3.88		8		
$-0.5 < Y < 0$	1.72	7.94	5	18	12.746
$0 < Y < 0.5$	2.34		5		
$0.5 < Y < 1$	5.67		4		.492
$1 < Y < 1.5$	11.25		14		.672
$1.5 < Y < 2$	15.67		10		2.052
$2 < Y < 2.5$	15.40		13		.374
$2.5 < Y < 3$	10.67		13		.509
$3 < Y < 3.5$	5.21		8		
$3.5 < Y < 4$	1.80		0		
$4 < Y < 4.5$	.44	7.53	1	9	.287
$4.5 < Y < 5$	.07		0		
$5 < Y$	.01		0		
Totals	132.00		132		20.346

principal component analysis) has a high predictive value with a probability, according to a chi-square test that this is due to chance, of less than .01.

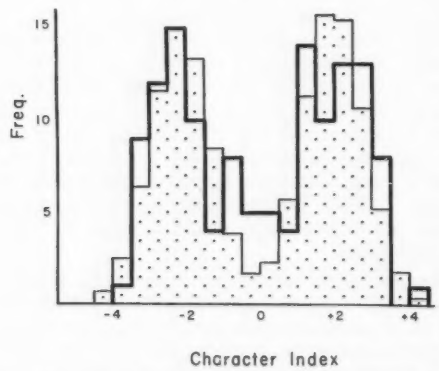

FIG. 2: Observed frequencies (clear histogram with heavy outline) and expected frequencies (stippled histogram) under the hypothesis that the distribution of the principal component character index is made up of two normal distributions with the same standard deviation; there is an excess of intermediate specimens.

Perhaps more importantly it supports our interpretation that some of the intermediate specimens are hybrids.

The intermediate patterns are related to the parental patterns in the same way that artificial F_1 hybrid muscle myogen pattern of salmonids (Tsuyuki and Roberts 1965) are, and that artificial F_1 hybrids between the redside shiner (*Richardsonius balteatus*) and the peamouth (*Mylocheilus caurinus*) are related to the parental patterns (Aspinwell and Tsuyuki 1968).

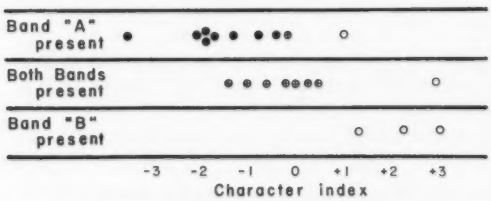

FIG. 3: Relations between the presence of electrophoretic bands "A" and "B", the numerical value of the principal component morphological character index, and preliminary identifications: ● = *Etheostoma nigrum*, ○ = *E. olmstedi*, ⊕ = intermediate specimen.

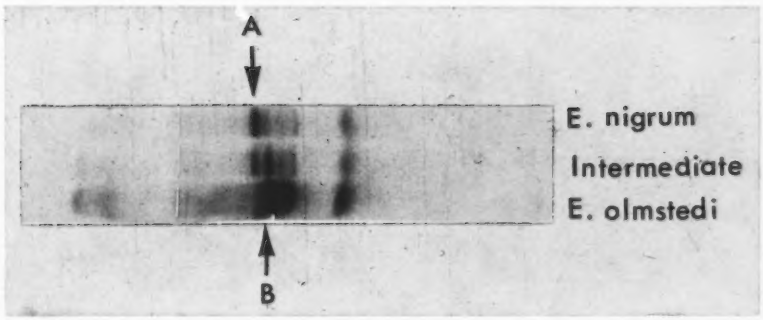

FIG. 4. Starch gel electropherograms of the muscle myogens of *E. nigrum*, *E. olmstedi*, and intermediates.

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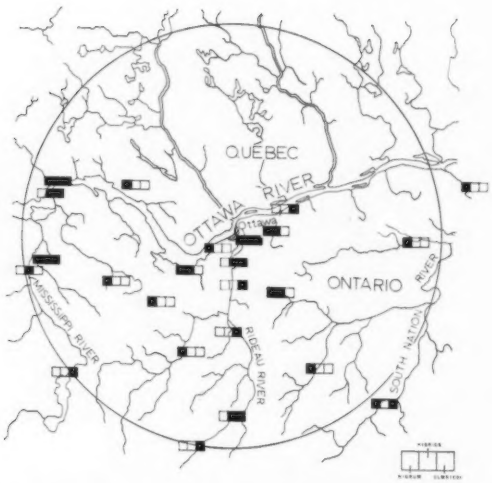

FIG. 5. Map of distribution of *E. nigrum*, *E. olmstedi*, and intermediates in the Ottawa area.

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Ecology

It is useful to examine biological features as well as purely morphophysiological characters in coming to a decision. Figure 5 shows the distribution of *nigrum*, the intermediates, and *olmstedi* in the Ottawa area. First it is clear that the two taxa are sympatric in the Ottawa area. Second, it appears that *olmstedi* occurs mainly in the three larger rivers of the area, the Ottawa, the Mississippi, and the Rideau, whereas *nigrum* occurs more frequently in smaller streams although also occurring in the Ottawa River. Hybrids occur either in the big rivers or in smaller streams. Examination of catalog sheets for the collections shows that *nigrum* tended to prefer small streams, those 15 m wide or less, whereas *olmstedi* tended to prefer rivers 30 m wide or more. *Nigrum* tended to live in faster water and more frequently on gravel or boulder bottoms, whereas *olmstedi* tended to live in slower or still water with sand, mud, or rock bottom. Thus, although these habitats were freely available to both species, each tended to select a distinct habitat. It seems likely that this habitat selection behavior was inherent. It is uncertain what effect environmental changes due to man (such as deforestation and dams) may have had on the degree of hybridization.

Discussion

The fundamental question is, to what do the three peaks of the character index correspond, 1) to two species and interspecific hybrids, or 2) to two subspecies and intersubspecific hybrids (intergrades).

The most essential feature of a species is the inherent discreteness of its gene pool (Bigelow 1965). From this feature flow all other general characteristics of species. It may achieve this by one or more behavioral, ecological, physiological or other isolating mechanisms, premating, mating, or postmating. These mechanisms may prevent hybridization with a related species. Hubbs (1967, p. 58), speaking of darters, stated that the capacity of two forms to produce hybrids does not by itself bear on their conspecific relation; it merely signifies that the parental taxa are relatively closely related. Gene pool purity may be maintained in the face of hybridization, if backcrossing is prevented or greatly reduced. Backcrossing may be prevented by the same kind of mechanisms that prevent hybridization.

We have previously shown that some of the specimens with intermediate character indices are probably hybrids. These are the 10 excess specimens in the three central classes of the character index, Table 5. The hybrid nature of some of these has been independently confirmed by the simultaneous presence of A and B myogen bands. The question remains, has there been backcrossing?

The removal of 10 excess specimens from the three central classes would reduced the chi-squared term total from 20.3 to 7.5, and lower the probability from $< .01$ to $> .05$. The deviation from the expected curve with the removal of the hybrids can then be attributed to chance. Because of the low number of hybrids the number of backcrosses would be small and difficult to detect in this sample. But if backcrossing had been going on, the parental peaks might then be expected to be significantly skewed towards the center. But we have just seen that the ("dehybridized") parental curves do not deviate significantly from normality. The amount of backcrossing and gene flow can therefore be said to be so small (if any) that it is not significantly changing the nature of the frequencies of character indices of the parental forms. The gene pools of *nigrum* and *olmstedi* in Lake Ontario drainages also appear to be discrete, there being no evidence of backcrossing in any character except possibly in the infraorbital canal (Cole 1965).

The broad zone of sympatry of the two forms (without swamping) in New York and Ontario is characteristic of species, rather than subspecies which are usually allopatric (Mayr 1969). The tendency of the two forms for different ecological preferences, is also more characteristic of species than subspecies (Gilbert 1961).

The absence of any significant gene flow between *nigrum* and *olmstedi* as tested in sympatric samples by examining morphological and myogen characters and the broad extent of their sympatry, in which they show different ecological preferences are characteristic of the species rather than the subspecies category. The johnny and tessellated darters are therefore judged, on present evidence, to be full species, *Etheostoma nigrum* and *Etheostoma olmstedi*.

For convenience of the reader, the most convenient and diagnostic of characters for the Ottawa Valley are summarized below in a key.

1 (2) Breast (especially), cheek, and nape usually scaleless. Complete bars crossing caudal fin usually 2–4. Soft dorsal rays usually 10–12. Upper lip usually even with tip of snout ..... johnny darter, *Etheostoma nigrum*

2 (1) Breast (especially), cheek, and nape usually with scales. Complete bars crossing caudal fin usually 5–8. Soft dorsal rays usually 13–15. Upper lip usually extending past tip of snout..... tessellated darter, *Etheostoma olmstedi*

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Mrs E. Roberts and Miss K. Whitlow assisted in electrophoretic analyses. Mr T. A. Willock assisted in making

some of the collections and counts. The research of one of us (P. J.) was supported by the National Research Council of Canada and le Ministère de l'Education du Québec. Drs C. C. Lindsey and C. G. Gruchy kindly criticized the manuscript. C. H. Douglas drew the map.

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Conversion, Maintenance, and Assimilation for Walleye, *Stizostedion vitreum vitreum*, as Affected by Size, Diet, and Temperature

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KELSO, J. R. M. 1972. Conversion, maintenance, and assimilation for walleye, *Stizostedion vitreum vitreum*, as affected by size, diet, and temperature. J. Fish. Res. Bd. Canada 29: 1181–1192.

An optimum ration was not readily apparent as conversion differed only slightly at high and low rations. Gross (K_1) and net (K_2) conversion was affected by neither ration size nor temperature, but decreased with increasing fish size. Conversion of the third order (K_3) was affected only by temperature, being greater at higher temperatures. Gross and net conversion were consistently lower at cycled temperatures, 16–8 C. Gross and net conversion were similar since the high assimilation efficiency (82.1–97.9%) of walleye (*Stizostedion vitreum vitreum*) did not greatly alter the latter.

Assimilation efficiency was dependent upon diet type, least for invertebrates (82.1% for amphipods and 83.5% for crayfish) and greatest for fish (96.9% for age O perch and 97.9% for emerald shiners). Assimilation efficiency decreased with increasing walleye size (slope of −0.0045) irrespective of the food organism. Regurgitation of crayfish occurred in two of three trials, and it was not established if this was a natural phenomenon.

Maintenance requirement per gram was independent of fish size, but was affected by temperature. Maintenance between temperatures of 4–12 C was similar, 33.3–45.7 cal/g walleye per week, but increased greatly above 12 C. Results from maintenance experiments paralleled Winberg's (1956) approximation of routine metabolism.

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La conversion ne différant que très peu à hautes et basses rations, il n'est pas facile d'établir une ration optimale. La conversion brute (K_1) et la conversion nette (K_2) ne sont pas affectées par le volume de ration ou par la température, mais diminuent avec la croissance du poisson. La conversion de troisième ordre (K_3) n'est affectée que par la température et augmente avec celle-ci. Les conversions brute et nette sont uniformément basses à des températures cycliques de 16 à 8 C. Elles sont semblables parce que la haute efficacité d'assimilation (82.1 à 97.9%) du doré jaune (*Stizostedion vitreum vitreum*) n'affecte que très peu la conversion nette. Le degré d'efficacité d'assimilation dépend de la nature du régime; elle est minimale avec des invertébrés (82.1% avec amphipodes et 83.5% avec écrevisses) et maximale avec des poissons (96.9% avec perches d'âge 0 et 97.9% avec ménés émeraude). A mesure que le doré jaune grandit, l'efficacité d'assimilation diminue (pente de −0.0045), quel que soit le type de nourriture. Au cours de deux ou trois essais, il y eut régurgitation d'écrevisses, et nous n'avons pu nous assurer qu'il s'agissait d'un phénomène naturel. Les quantités nécessaires à l'entretien, par gramme, sont indépendantes de la taille du poisson, mais sont affectées par la température. Elles sont les mêmes sur une gamme de températures de 4 à 12 C, soit de 33.3 à 45.7 cal/g de doré/sem, mais augmentent considérablement au-delà de 12 C. Les résultats de nos essais sur les rations d'entretien sont en accord avec l'approximation du métabolisme de routine donnée par Winberg (1956).

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ACCURATE assessment of fish growth and a knowledge of the disposition of ingested nutrients are essential to the management of any fishery. In an analysis of an unexploited walleye (*Stizostedion vitreum vitreum*) population (Kelso and Ward 1972) it was found that older fishes contributed less to biomass and production than newly recruited individuals. To obtain insights into the observed walleye production of West Blue Lake a series of experiments were conducted on the basis of theoretical reviews

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of growth and metabolism (Paloheimo and Dickie 1965, 1966a, b).

Since Paloheimo and Dickie's reviews were not based on their own experimentation, the following experiments were conducted (a) to assess growth mechanisms in the walleye, a terminal member of the trophic structure and (b) to examine the applicability of the theoretical reviews. The calorie was used as the unit of measure, thus avoiding difficulties of approximation.

Methods and Materials

Capture, Transport, and Holding

Walleye, age II–VI years, were captured from West Blue Lake, Man., in a standard gang (Kelso and Ward 1972) from May 22 to 26, 1970, and kept in 560-liter tanks on the lakeshore. On May 26, walleye were anesthetized (M.S. 222 at 1:75,000) and transported to the laboratory in two plastic tanks of 455– and 270-liter capacity. Water was maintained between 6 and 8 C by ice. Oxygen was kept at air saturation using an aerator operating on a 12-v wet cell battery. On arrival, all fish were placed in one 2730-liter fiberglass tank and held at 8 C.

Incoming domestic water is passed through activated charcoal filters to remove chlorine and is available at 3.3, 11.7, and 26.7 C. All plumbing for the system is PVC (polyvinylchloride). Air saturation with oxygen is 100% (3.3 and 11.7 C lines) and 85% (at 26.7 C), while alkalinity is 120 mg/liter and *p*H varies from 8.5 to 8.9.

Temperature control was achieved using a Power Series 440 Fotoguard mixing valve modified to integrate any of the three supply lines. Temperature adjustments were made, if necessary, twice daily. Experimental animals were subjected to a diel cycle of 14 hr light and 10 hr dark. In experiments where temperature was cycled, changes were coincident with light alterations. Except when stated, 560-liter fiberglass tanks covered by a translucent green fiberglass cover were used.

To minimize the incidence of infection, malachite green, at an initial concentration of 1:150,000, was administered to walleye soon after their arrival at the laboratory.

After holding for 1 week at 8 C, food in approximately 100-g units was placed in the tank once daily. Surplus food was removed after 1 hr. This practice continued until most fish readily accepted the food. Those individuals not under experimental conditions were kept at 12 C and fed at 3% of body weight per day.

Conversion and Maintenance Experiments

Food for maintenance and conversion experiments was the emerald shiner, *Notropis atherinoides*, captured by seining at Delta Marsh on Lake Manitoba on May 14–16, 1970. Most of these minnows were frozen in sealed, sterile, plastic bags. Several thousand were maintained live as food for age III+ and older walleye. Every 2 weeks a sample of the frozen food was analyzed to determine any change in caloric content.

Individual walleye in an experimental group differed by no more than 10 g live weight.

Walleye were acclimated from 12 C to the experimental temperature at the rate of 1 degree change per day, then were subjected to experimental conditions of food and temperature for 2 weeks before an experiment was considered started.

Frozen emerald shiners were given to all II+ fish in growth and maintenance experiments. Food was partially thawed, counted when amounts introduced were less than 20 g, and weighed. Walleye age III+ and older refused to accept dead food; therefore, in size comparisons for maintenance requirements, food was live emerald shiners of known energy content. Live weight was obtained by placing minnows in a tared 500-ml beaker containing aerated tank water. All walleye were fed 6 days per week at the rate of one food lot per day.

For conversion experiments, food was introduced daily. Any excess was removed and weighed after allowing 4 hr for walleye to feed. In maintenance studies, food was adjusted every 2 days if a gain or loss in weight occurred in experimental animals.

Overflow of all tanks was screened by a double layer of nylon seine net to trap all excess food. Screens were removed, cleaned, and any unused food was weighed.

Since fish were fed 6 days per week, weights were obtained on the seventh day. Before weighing, each animal was anesthetized in 25‰ M.S. 222, measured, and then blotted using paper towels.

Availability of the Ration

To assess assimilation (also called availability in this paper) of major natural items of the diet, walleye were acclimated to 12 and 20 C and isolated in 40- and 220-liter glass aquaria. Large food items (perch, *Perca flavescens*, and crayfish, *Orconectes virilis*) were manually placed in the pharynx where they were voluntarily swallowed. Smaller organisms (*Gammarus lacustris* and emerald shiners) were introduced into the stomach by a force-feeder consisting of a plunger in a smooth Tygon plastic tube. All food except emerald shiners was obtained from West Blue Lake.

Initial experiments defined the time between ingestion and egestion; therefore, individuals were continuously observed at the time of expected feces emission. Feces were collected within an hour of emission using a large pipette, strained through a 53-micron copper sieve, and oven-dried at 105 C to a constant weight. Filtration of aquarium contents indicated that feces collection was complete.

Calorimetry

All samples used to determine caloric content were weighed live (wet weight), then dried at 105 C to a constant dry weight. Duration of drying was from 3 (for small organisms) to 8 days (for adult walleye). If storage after drying was necessary, samples were placed in dessicators with either silica gel or phosphorus pentoxide (P_2O_5) as desiccants.

Dried walleye were pulverized individually in a blender until homogeneous. Approximately 50 g was then redried. Each estimate for caloric value of walleye tissue was the mean of two determinations. If values for the two combustions differed by more than 125 cal/g, an additional sample was burned. From one walleye pulverized in the blender, five separate samples were combusted to define the degree of homogeneity attained by this procedure. Smaller organisms were ground with a pestle to a fine powder in either a porcelain or agate mortar.

Organisms yielding 0.75 g or more of dried material were combusted in a recently standardized Gallenkamp automatic adiabatic bomb calorimeter that was calibrated weekly using benzoic acid as a standard.

Other organisms were combusted in a Phillipson (1964) microbomb calorimeter supplied by Gentry and Wiegert Instruments, Inc. This instrument was standardized weekly using benzoic acid as a standard.

To obtain the percent ash content of samples, dried material was placed in pre-combusted, aluminum foil weighing dishes and heated at 500 C for 24 hr.

Experimental Approach

The basic energy equation for an individual or a population may be written as $\frac{\Delta W}{\Delta t} = pR - T$, where the energy deposition in terms of growth during any time Δt ($\frac{\Delta W}{\Delta t}$) is the resultant of the ration R (corrected to that which is available by pR) and the total metabolic expenditure T. It has been shown (Paloheimo and Dickie 1965) that $T = \alpha W^{\gamma}$ and $\log T = \log \alpha + \gamma \log W$ where W is the weight, α is a constant defining the level of metabolic expenditure, and γ defines the rate of metabolic expenditure with weight. These relations, discussed in detail by Paloheimo and Dickie (1965), are the basis for this description of energy transformation by the walleye. Throughout this study the coefficient p for availability of the ration is determined as $\frac{\text{intake (cal)} - \text{feces (cal)}}{\text{intake (cal)}}\%$. Winberg (1956) states that disregarding excretion of soluble wastes results in an error of less than 3% of the energy consumed.

Efficiencies of growth (K) for a particular species can be determined by comparisons of growth to intake. Several expressions of conversion are possible: $K_1 = \frac{\Delta W}{R\Delta t}$, which relates the weight gained per unit time ($\Delta W/\Delta t$) to ration size (R), and $K_2 = \frac{\Delta W}{pR\Delta t}$, which relates weight gained per unit time to the available ration (pR). These relations are called either growth coefficients *of the first and second order, or gross and net conversion,* respectively. In addition, the metabolic expenditure T can be obtained by $T = pR - \frac{\Delta W}{\Delta t}$. In maintenance studies where $\frac{\Delta W}{\Delta t} = 0$, $T = pR$. Since T also equals αW^{γ}, the parameter α and γ can be obtained from nutritional studies. All energy ingested is not available for growth, therefore $K_3 = \frac{\Delta W}{pR\Delta t - T_R}$ eliminates the routine metabolic requirement T_R (which equals αW^{γ}. from maintenance experiments). The latter expression of conversion is the growth coefficient of the third order.

Results

Emerald shiners, frozen or alive, had energy values of 4964 cal/g (standard deviation ± 68); 12.6% of dry weight was ash, and 75.9% of the live weight was removed on drying (28 determinations made during 1–6 months of storage). Because of the narrow statistical limits, energy content of the food was considered constant.

Analysis of eight walleye captured May 24, 1972, indicated that caloric content was homogeneous, 4580 ± 68 cal/g (Kelso MS 1972). Investigations into growth and maintenance began 3 weeks after arrival at the laboratory; thus, caloric changes in walleye were assumed to be minor.

In any experiment where consumption was above the maintenance ration, only age II^{+} individuals could be utilized. Older walleye, aged III–VI years, would consume a minimal amount of live food, then cease feeding.

To assess accuracy of caloric examination, one walleye, age IV^{+}, was subsampled five times and a 1 g sample combusted from each. From this replicate series, the mean energy of this fish was found to be 5005 cal/g dry weight (range 4975–5042 cal/g) with a standard deviation of 26.4. It was apparent that the variability within a sample is small and within the stipulated capacity of the Gallenkamp calorimeter.

Effect of Ration Size on Conversion

To establish the effect of ration size (R) on conversion efficiency, groups of fish were fed for 2 weeks on rations of 2.48–8.53% body weight (Table 1). The range in conversion efficiencies (K_1) was relatively narrow, 0.106–0.142; however, there appeared to be a slight optimum at rations of approximately 4%. Net conversion (K_2) followed a similar pattern. Two separate determinations were made near a 4% ration, but conversion was different at each. The two rations differed by only 0.2% of body weight, but conversion efficiencies differed by 1.5%. The observed difference was almost certainly not the result of walleye weight differences. Nevertheless, it seemed that at higher rations, greater than 6% of body weight, gross conversion efficiency was reduced. At moderate ration sizes, 3.3-4.3%, conversion was within a relatively narrow range (0.129-0.142). Only one ration of relatively small

TABLE 1. Effect of ration size (% body weight) on conversion (K_1 and K_2) for walleye held at 16 C. Fish were fed at similar intakes for 2 weeks.

No. of animals	Intake (R) (*cal*)	Growth (*cal*)	% Body Weight	*p*R	Maintenance for week	K_1	K_2	Mean Walleye size (g)
3	86,218	10,554	2.50	83,028	31,560.3	0.122	0.128	160.12
	87,578	10,594	2.48	84,318	32,104.3	0.121	0.126	162.88
3	142,902	18,778	3.32	137,615	39,377.4	0.131	0.137	200.09
	146,674	19,096	3.32	141,248	40,405.5	0.131	0.135	205.00
3	142,902	18,515	3.98	137,615	32,866.6	0.130	0.135	161.84
	144,292	18,629	3.96	138,376	39,062.2	0.129	0.135	166.75
3	153,276	21,827	4.16	147,605	28,831.8	0.142	0.148	136.94
	153,326	21,814	4.17	147,599	30,014.4	0.142	0.149	146.28
4	271,261	34,430	6.16	261,224	39,034.2	0.127	0.132	146.52
	261,185	34,188	5.69	251,521	40,741.8	0.131	0.136	153.27
4	393,415	42,371	8.53	378,859	43,473.9	0.108	0.113	162.39
	391,380	41,010	8.20	376,899	45,522.2	0.106	0.109	170.70

size was administered (2.49% of body weight); therefore, the effect of low rations on conversion could not be fully defined. Apparently not much difference in conversion existed for rations up to 4% of body weight.

CONVERSION

To assess conversion, a ration (4% body weight) was administered to groups of walleye held at temperatures within the range encountered by fish in West Blue Lake. Growth was active and relatively homogeneous throughout the experiment (4–13 weeks). Walleye weight changes and consumption were treated as being cumulative for initial inspection. Walleye weight gain (Fig. 1) seemed greatest for animals held at 20 C. Fish used in all growth studies grew at similar rates, but the caloric conversion of ration to growth was of most interest.

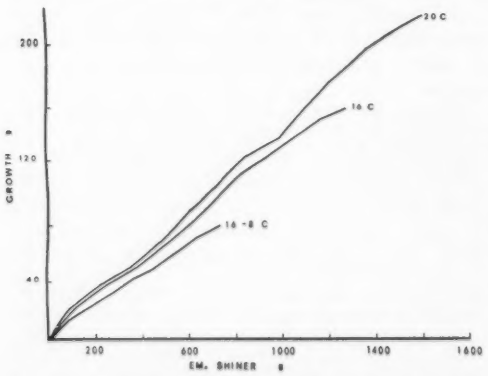

FIG. 1. Accumulation of weight (*g*) by walleye fed 4% body weight at 20, 16, and 16–8 C.

Although experiments began at approximately the same time, and the animals were therefore assumed to be of one energy content (4580 ± 77 cal/g), variation in the duration of experiments affected the terminal caloric values (Table 2).

Gross energy content varied with trial duration. Also, the weekly gain (Table 2) was relatively constant for each experiment. The final caloric values for the variable temperature (16–8 C) and 12 C series were not best estimates since the variability associated with the former experiment was great, and the latter resulted from only one determination. Nevertheless, the relatively constant weekly calorific gain, indicated by before and after measurements, were supported by evidence from the field (Kelso MS 1972).

Weekly values of gross conversion $\left(\frac{\Delta W}{R\,\Delta t}\right)$ during experiments of 8–13 weeks were extremely variable (Fig 2). Experiments at 20 C indicated a general decrease in growth efficiency with trial duration. On the other hand, gross conversion at other temperatures was either variable (16 C) or quite static (16–8 C variable day cycle). Gross conversion in walleye ranged from about 0.100–0.160. Estimates of K_1 conversion (Table 3) for the entire duration of each feeding trial was 0.143 ± 0.020 (20 C), 0.127 ± 0.021 (16 C), and 0.136 ± 0.018 (12 C). The cycled temperature series (16–8 C) yielded a lower estimate of K_1. Gross conversion calculated for the three stable temperature experiments (Table 3) were not significantly different (F value 2.036 ns).

Conversion efficiency that includes the energy available to an individual (pR) must take into account the effect of fish size on the assimilation coefficient (p) of a ration. Assimilation efficiencies for emerald shiner were derived (presented subsequently) for sizes of walleye and were applied weekly to the ration ingested in conversion experiments. Net conversion $\left(\frac{\Delta W}{p\ R\Delta t}\right)$ was only slightly higher than gross conversion (K_1) (Table 3, Fig. 2) because of the high efficiency of food utilization by walleye — maximum efficiency on emerald shiner diet of 97.9%. However, since assimilation decreased with increasing fish size, estimates of net conversion (K_2) were increased very slightly for the latter conversion estimates of each feeding trial (Fig. 3). Generally, conversion efficiencies decreased with increasing mean walleye weight (Fig. 3). Decreased assimilation efficiencies occurring in the latter parts of feeding trials did not alleviate the apparent effect of weight. Variability in conversion in the 16 C experiment was still evident. Analysis of variance among K_2 values at stable temperatures indicated no significant differences (F value 2.024 ns). Once again, mean net conversion at the variable temperature (Table 3) was considerably lower (0.117) than conversion at stable temperatures.

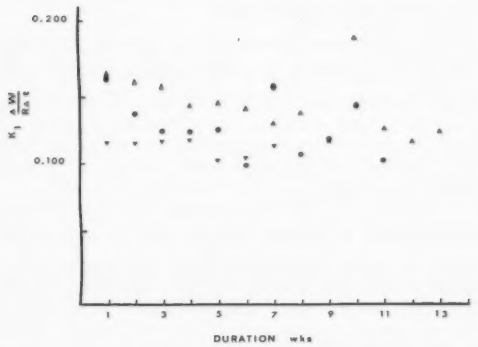

FIG. 2. Effect of experiment duration on K_1 for walleye held at 20(△), 16(●), and 16–8 C (▼).

The conversion coefficient of the third order, K_3, eliminates the routine expenditure (T_R) which is temperature dependent and is approximated by $T_R = 0.3W^{0.8}$ (Winberg 1956). Maintenance requirements for walleye of various sizes were established (presented later) and a log-logplot through these requirements, plus those of one size at several temperatures corrected to 20 C (Krogh's temperature correction from Winberg), described the level of metabolism, $\alpha = 0.301$ mg O_2/hr, and the weight exponent, $\gamma = 0.841$. Because of the similarity

TABLE 2. Change in energy content of whole, age II+ walleye during conversion experiments (standard deviations are in parenthesis).

Start			End			
Experiment	Duration (*weeks*)	Cal/g	No. analyzed at end	Cal/g	% Moisture	Weekly caloric gain
20C	13	4580(77)	2	5043(56)	74.72(0.79)	36
16C	11		2	4937(30)	74.47(0.20)	32
16-8C	8		2	4856(130)	74.63	35
12C	4		1	4722	74.54	35

TABLE 3. Conversion efficiencies (standard deviations are in parenthesis) of age II+ walleye for experiments at different temperatures.

Temperature (*C*)	K_1	K_2	K_3	Experiment duration in weeks
20	0.143(0.020)	0.149(0.021)	0.229(0.031)	13
16	0.127(0.021)	0.132(0.021)	0.173(0.034)	11
16–8	0.113(0.006)	0.117(0.006)	0.134(0.006)	8
12	0.139(0.018)	0.145(0.018)	0.169(0.023)	4

of these two estimators of routine expenditure, Winberg's (1956) generalized formula was corrected to experimental temperatures and used to determine K_3.

At 20 C, K_3 values were approximately linear throughout the experiment duration (Fig. 3) and were not affected by weight changes. The linear regression for the relation of K_3 values on walleye size was not significant ($H_O:\beta = 0$, $F = 0.03$ ns). Variability in the magnitude of K_3 still occurred at 16 C. Examination of the mean K_3 values from the three stable temperatures (Table 3) revealed a significant difference (F 15.595, $P > .05$). Tukey's w-procedure for determining means not significantly different (5% level):

Experimental Temp.	20 C	16 C	12 C
K_3 conversion	0.229	0.173	0.169

indicated that a similarity of conversion existed only at adjacent temperatures. As with K_1 and K_2 conversion coefficients, K_3 conversion for the 16–8 C variable temperature experiment was less than at stable temperatures.

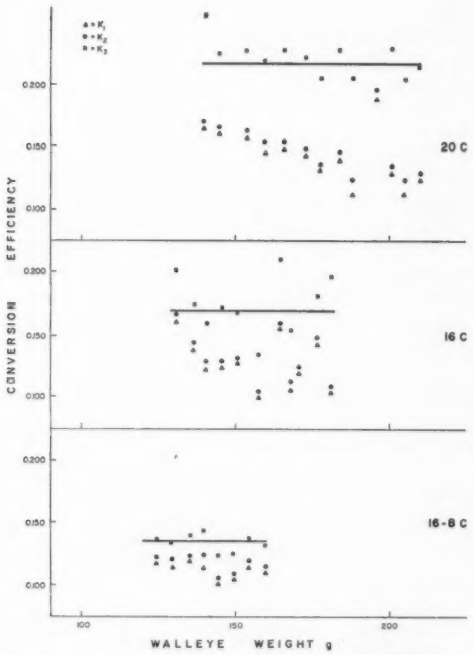

FIG. 3. Conversion efficiencies in three orders (K_1, K_2, and K_3) as affected by walleye size.

MAINTENANCE REQUIREMENTS

Maintenance requirements for walleye depended essentially upon fish size and ambient temperature. Table 4 shows the requirements at 12 C for II, IV, V, and VI year old fish. Requirements per gram of weight were similar regardless of specimen size and varied within a narrow range, 36.5–38.2 mg/g per week.

Since caloric loss or increase may occur in walleye, independent of weight changes, age II individuals were analyzed calorically after two maintenance trials conducted at 8 and 20 C. Once again, 4580 cal/g dry weight (±77) was considered as the initial caloric value. At the end of the two trials, caloric content of walleye was 4608 and 4611 cal/g for 8 and 20 C experiments, respectively. Variation within all trials was not determined, since the number of experimental animals was limited.

Since the energy required per unit weight for maintenance of walleye was almost independent of size, three walleye at 4 and 8 C and two individuals at 12, 16, and 20 C were fed maintenance diets to determine temperature effects. Requirements were converted from a gram/gram basis to cal/g and indicated a rapidly increasing demand from 12 to 20 C (Fig. 4). At the three lowest temperatures (4, 8, and 12 C) the maintenance requirements varied only slightly, 33.25–45.70 cal/g per week. Temperature was also cycled, 16–8 C, in one maintenance trial for a period of 4 weeks. The requirement was 40.2 mg/g per week and exceeded the normal requirement at a stable temperature of 12 C (Fig. 4).

TABLE 4. Maintenance requirements expressed as mg/g per week of II, IV, V, and VI year old walleye at 12 C. Three fish were used in each determination.

Mean walleye size (g)	Experiment duration (*weeks*)	Average weekly consumption (g)	mg/g per week requirement
169.56	4	12.72	38.2
476.77	3	33.83	36.5
682.72	3	49.36	36.7
889.05	3	66.21	37.3

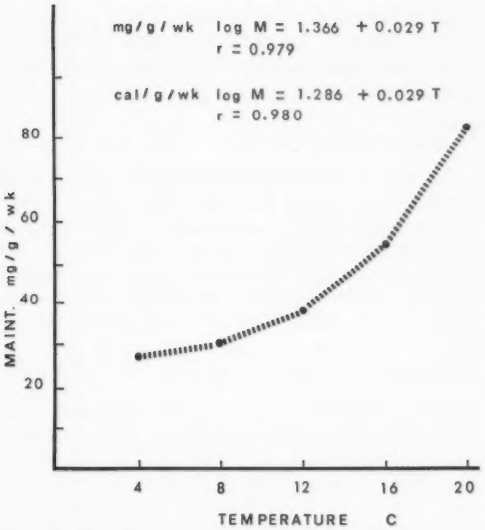

FIG. 4. Relation between maintenance requirements and temperature for walleye.

In maintenance trials, weight increase $\left(\frac{\Delta W}{\Delta t}\right)$ was zero and the total metabolic expenditure (T) is the available ration (pR). Since maintenance was determined for various sizes of walleye and for age II fish at several temperatures, all measures were converted to 20 C by Krogh's correction (Winberg 1956). A log–log description of log T = 252.3 + 0.841 log W described adequately the relation between walleye weight and routine metabolism. The level of metabolism, 252.3 cal., when converted to mg O_2/hr is 0.301. In this relation, 87.2% of the variability ($r^2 \times 100$) in the dependent variable can be attributed to the independent variable (weight).

ASSIMILATION

Most foods were egested as a solid streamer of waste; however, when fed crayfish, experimental animals retained the ingested material for approximately 10–16 hr and then regurgitated the carapace intact, but empty of soft tissue, along with partially emptied abdominal segments. Three trials (for a total of 15 walleye) were conducted using crayfish, and the observed phenomenon occurred in two trials (10 fish). Only the latter trial was considered in analysis as it was not apparent whether regurgitation of crayfish is a natural phenomenon. Fecal material from animals that had regurgitated was not detectable. Time required to egest a meal of crayfish varied from 38 to 72 hr for those that had retained the entire meal. The amphipod, *Gammarus lacustris*, had the shortest residence time in the gut, from 28 to 36 hr, and appeared in feces with exoskeletons intact. Parts of the exoskeleton (legs and abdominal portions) were fragmented, but fecal contents were readily recognizable as amphipods. Unidentified remains of both emerald shiners and age 0 perch were egested 48–56 hr after ingestion.

Caloric content of fecal material was generally low, 1099–2979 cal/g dry weight. Ash content of fecal materials was high, but since only about 40% of ash ingested appeared in the feces, neither ingested nor fecal calories were corrected to cal/ash-free gram. Only 11 comparisons of ash in feces as a percent of that ingested by walleye in assimilation studies were available, but they did indicate considerable utilization of material remaining as ash on incineration at 500 C:

Walleye size	No. determinations	% ash in feces
II+ 113.2–173.3g	7	42.7(± 7.26)
IV, V 360.4–476.8g	4	38.5(±19.22)

Only age 0 perch of the larger ration sizes provided sufficient feces for combustion and ash determination.

Since efficiency of utilization may be affected by the meal size ingested, rations of age 0 perch ranging from 7.80 to 44.91 cal/g — 0.7 to 5.2% body weight — were administered to walleye of various sizes (Fig. 5). Ration sizes much larger than 5% of body weight could not be administered since increased handling, associated with force-feeding of small organisms to walleye, was detrimental to experimental animals. For the range of ration sizes used, assimilation efficiency was constant (Fig. 5) within age II and within ages IV and V. The slope of each regression line was zero (H_0:$\beta = 0$); F value for age II walleye was 0.044, and for older fish, 3.33. The difference in efficiencies of the older fish (Fig. 5) was related to fish size, as fish of approximately 360 g were more efficient than walleye of 500 g.

Walleye of various weights, ranging from 113.2 to 502.0 g, were fed rations between 7.80 and 11.06 cal/gram. Assimilation efficiency decreased with increasing fish size, E = 96.851 – 0.0045 W, where E is the assimilation efficiency and W is fish weight (Fig. 6). The regression coefficient was significant (Table 5) and 82.4% of the variability in assimilation efficiency was attributable to weight changes.

To determine if this relation applied to other diet types, walleye of different sizes were fed amphipods in amounts of 8.21 to 11.17 cal/g. This relation, E = 82.103–0.0041 W, was also highly significant and r^2 was 0.859. Regression coefficients from assimilation equations of both age 0 perch and amphipods were tested for homogeneity (H_0:$\beta_1 = \beta_2$). The hypothesis was accepted ("t" = 0.418 ns) and slopes were assumed to be similar. The obvious difference in intercepts (Table 5) reflected the utilization of various food types. The effect of fish size on assimilation efficiency was not determined for all food types. The two regression lines (Table 5) for crayfish and emerald shiners were derived by solution using a "b" value of 0.0045. The slope from the age 0 perch trials was utilized since more experimental animals were available in that assessment (14 walleye). To solve for the intercepts (a) of Table 5, four walleye of similar size were fed known amounts of food and results were combined to provide a better value for assimilation by a particular fish size.

Energy for Metabolism

All fish in conversion experiments were fed at similar rates (approximately 4% body weight), but had differing K_3 conversions. It was apparent that the excess energy, above that required for growth and routine expenditure (from maintenance studies), increased with decreasing temperature (Table 6). A portion of the total metabolic expenditure $\left(T = pR - \frac{\Delta W}{\Delta t}\right)$ must be utilized for routine metabolism ($T_R = 0.3W^{0.8}$). If the active metabolic expenditure, taken as $2T_R$ (Winberg 1956), was applied to fish at 16 and 12 C, the requirement is greater at higher temperatures. However, it is apparent (Table 6) that the expenditure (ΔT) at 20 C is considerably less than at 16 and 12 C. In addition, the expenditure in the variable temperature regime is intermediate to that of the

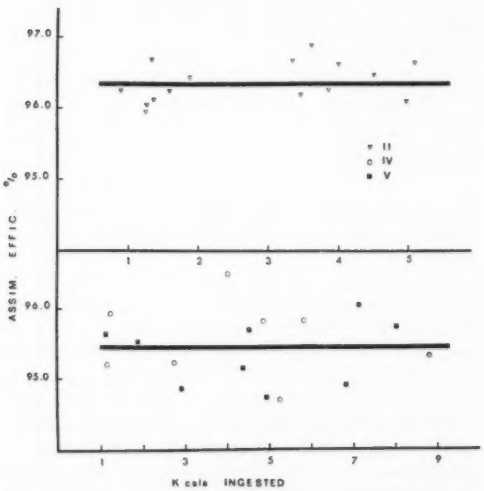

FIG. 5. Effect of ration size (Kcals ingested) on the assimilation of age 0 perch by walleye aged II+ (upper) and older (lower) at 16 C.

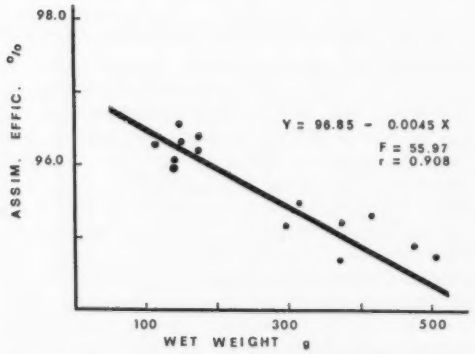

FIG. 6. Effect of walleye size (*g wet weight*) on assimilation efficiency at 16 C.

TABLE 5. Experimental and derived (equations for crayfish and emerald shiners derived by procedures in the text) relations between assimilation efficiency (E) and walleye weight (W) for various natural foods.

Food	Regression	No. observations	F	r^2
age 0 perch	E=96.851–.0045W	14	55.97	0.824
amphipods	E=82.103–0.0041W	8	33.79	0.859
crayfish	E=83.535–0.0045W	4		
emerald shiner	E=97.871–0.0045W	4		

TABLE 6. Weekly energy utilization by walleye for maintenance, routine metabolism (T_R) and the excess in energy (ΔT) above twice the routine level ($T-2(T_R)$). All measures are in calories and figures in parenthesis are standard deviations.

Temp. (C)	$T(=pR-\frac{\Delta W}{\Delta t})$	Maintenance (Kelso MS 1972)	$2(0.3W^{0.8})$ (Winberg 1956)	$\Delta T=(T-2(T_R))$
20	121,206	45,141	94,581	27,318
	(7743)	(3147)	(9654)	(9322)
16	115,607	33,887	60,217	55,390
	(12,180)	(3207)	(5194)	(14,090)
16-8	93,631	13,261	25,311	68,320
	(5849)	(1133)	(1720)	(5984)
12	121,769	21,127	39,381	82,388
	(11,271)	(1024)	(1534)	(10,599)

two adjacent stable temperatures. This energy "excess," ΔT, cannot be further apportioned without additional experimentation.

Discussion

The unit of measure in the assessment of requirements, expenditures, growth, and efficiencies in fishes is controversial. Protein has been considered as the primary measure of growth (Gerking 1952, 1954, 1955); yet energy, for metabolism at least, is derived from all nutritional sources. Protein is undoubtedly an excellent index of growth alone, but nutritional analysis must be in a form that considers all energy sources. Caloric flow, if assessed, has usually been derived from measures of fat, protein, and carbohydrate. Since relative proportions of carbohydrate, fat, and protein are in a labile and dynamic state — deaminated proteins can be converted into fats and/or carbohydrates (Baldwin 1952) — the overall utilization of food can be most readily expressed in caloric terms.

Caloric content (or nutrient composition) of experimental fish has not usually been examined during feeding studies — notable exceptions being Menzel (1960) and Brett et al. (1969). The assumption that caloric content in walleye was homogeneous at the beginning of laboratory holding was realistic since West Blue Lake walleye exhibited consistent, age-independent, energy content when experimental animals were captured (Kelso MS 1972). Caloric uptake in fish flesh was considerable in this study (Table 2), but this phenomenon has apparently not been of great import to previous investigations. As well, all fish within conversion studies grew at similar rates, indicating that group feeding, in this instance, was not detrimental to experimentation.

Temperature has been known to influence the amount of food consumed by laboratory fishes (Hathaway 1927; Baldwin 1956; Bridges 1961). Intake in walleye at temperatures less than 12 C was insufficient to produce detectable growth. Below 12 C, intake was sufficient only for maintenance requirements. Moreover, since seasonal differences in stomach contents of fishes occur (Priegel 1963; Lawler 1965; Northcote and Lorz 1966; Kelso MS 1972) but do not generally fulfill the capacity of the organism to feed, and since feeding at extremes may alter conversion by depressing assimilation, moderate rations were considered a better approximation of natural processes. Furthermore, conversion was found to be constant for walleye within a narrow range at lower rations (Table 1).

As walleye undergo diel migrations, temperatures of hypothetical locations (thermocline and inshore regions) in West Blue Lake were applied to the photoperiodic cycle to assess the interplay of conditions during natural feeding. Variable temperatures produced results dissimilar from stable conditions (Fig. 2 and 3, Table 3). All estimates of conversion (K_1, K_2, and K_3) were lower in the variable temperature experiment. After removal of the routine requirement from the ingested ration $\left(K_3=\frac{\Delta W}{pR\Delta t-T_R}\right)$ conversion was still lower, indicating that a compensatory mechanism requiring energy is in action.

In almost all cases, only two forms of conversion have been considered. Johnson (1966) utilized the maintenance requirement in establishing conversion of pike, but had not located the available ration. Brody (1945) and Brown (1946, 1957) also utilized the maintenance fraction, but it will become evident that the K_3' descriptions of efficiency are even more enlightening for descriptions of food utilizations.

Brett et al. (1969) found an "optimum" ration for young sockeye, but in this instance an optimum conversion or ration was not readily apparent (Table 1). However, the observed relation may result from experimental variability alone. Differences in conversion for walleye at rations between 2.4 and 6.0% body weight (at 16 C) were slight (Table 1). Further trials to establish the presence or absence of an optimum conversion would have been desirable. Time and experimental animals were limited since all trials were conducted coincident with natural growth.

Paloheimo and Dickie (1966b), in their summary of available literature, found that increasing rations generally decrease growth efficiencies (log K_1). My results indicate that conversion efficiency, K_1 and K_2 (Fig. 3), was related to body size, although not to ration size, for the weight-proportional rations. Fig. 6 indicates that the effect may be related to changing assimilation efficiency; however, K_2 still exhibits a negative slope. Kerr (1971) concluded that in laboratory experiments spontaneous activity must be a major factor accounting for the slope of Paloheimo and Dickie K-lines. From a comparison of the K_1 and K_2 coefficients with K_3, it is herein apparent that some element of activity, included in routine metabolism (T_R), must be involved in the efficiency changes.

Direct conversion of ingested material into fish flesh is quite low in walleye, 0.10–0.16, and can be considered independent of temperature at any one ration size (Table 3). Net conversion is slightly higher, but the assimilation of an ingested meal in walleye is so efficient (95% or higher for emerald shiners; Fig. 5, Table 5) that gross conversion is only slightly less than net conversion (Fig. 3). Menzel (1960) found gross conversion of 0.16–0.25 for a reef fish, and also found that assimilation was between 89 and 98%. Both conversion and assimilation (Menzel 1960) were temperature independent and, thus, are in general support of the results of this investigation. Pandian (1967) found conversion of 15% in 150-g fish, similar to the weight and conversion efficiencies of this study, and stated that other researchers found conversion to be as low as 15% for fish of similar size. Low efficiency in walleye may be peculiar to its trophic level position. On the other hand, low conversion may result from stress applied through handling, incomplete assessment of assimilation, activity related to feeding level, or activity resulting from the mild surface currents created by incoming water. Two possibilities would be of minor importance since feces collection was similar to Menzel (1960) and relatively efficient, and currents did not generate appreciable activity.

Generally, maintenance ration in feeding trials reflects the energy required for activity, respiration, tissue replacement, restoration of mucous coats, etc. The logarithmic presentation of the relation between body weight and maintenance ($T = pR$ corrected to 20 C by Krogh's correction) for walleye is in excellent agreement with Winberg's (1956) generalized equation of $T = 0.3W^{0.8}$. Winberg's approximation has been supported by respirometry of various species of freshwater fishes. Mann (1965) and Job (1960) have previously indicated the agreement between nutrition and respirometry studies.

Rates of gastric digestion have been shown to be temperature dependent, generally decreasing at lower temperatures (Molnar and Tolg 1962; Seaburg and Moyle 1964). However, temperature has no effect on the assimilation efficiency of walleye. Feeding at low temperatures was sporadic, but clearance rate had no apparent effect on efficiency. The remarkable efficiency of fishes in assimilating food has been verified (Gerking 1955; Menzel 1960; Pandian 1967). Walleye are 82–98% efficient in assimilating various rations (Table 5). Only solid egesta was considered; Winberg (1956) states that loss in soluble form is less than 3% of ingested ration. Efficiency is dependent upon food species consumed and size of walleye.

Walleye also appear able to utilize some of the ash fraction of ingested material (see text table). This phenomenon seems not to have been detected in any other case, but does seem logical since material such as $CaCO_3$ is readily solubilized in an acid medium.

Ration size seems to have little direct influence on assimilation in walleye (Fig. 5); however, increasing weight decreases efficiency (Fig. 6). Menzel (1960) found that neither temperature nor fish size played a significant role in assimilation of the red hind (89–98%). As well, Gerking (1955) found efficiencies, in the range of 95.8–97.6% for protein, which were not affected by meal size, but did not examine the effect of fish size on assimilation.

Pandian (1967) found that neither temperature nor fish weight play a part in assimilation efficiency. The lower efficiency for assimilation of invertebrates by walleye (Table 5) no doubt results from the exoskeleton, which contains large amounts of chitin, not considered digestible by fish (Gerking 1952).

Since maintenance requirements were established, approximations of metabolic expenditures were determined from these nutritional studies (Table 6). Sufficient energy was available for activity and other bodily functions not examined by this report. As well, the metabolic approximations indicate

that no abnormal energy-demanding stresses were placed on experimental animals.

Maintenance at the variable temperature, 16–8 C, was slightly higher than at the mean temperature, 12 C, of the series and indicates that when exposed to a naturally changing water temperature (coincident with movement to and from inshore areas) energy is required. This phenomenon is supported by the apparent effect of cycled temperature on conversion (conversion was always lower at cycled temperatures). No information is available to support this claim as respirometry, as well, has been performed in stable temperatures with acclimated subjects.

These investigations on walleye have defined growth, assimilation, and a maintenance requirement. Obviously an excess remains (from approximately 15–65% of the ingested ration). Since all fish were on a 4% ration at all temperatures, it is evident (Table 6) that the excess energy (ΔT) increases at lower temperatures. This phenomenon occurs, although is not stated as such, in other studies as gross and net conversion have been shown to be temperature independent for similar ration sizes. Some mechanism (increased specific dynamic action at lower temperatures; increased excretion in some form at lower temperatures; or, improbably, an increased metabolism at lower temperatures) must either use or void this energy excess. No explanation as yet can be soundly based even though this fraction represents a major portion of ingested rations.

Warren and Davis (1967) have also found a metabolic excess for fish fed similar amounts at stable temperatures. In their work on *Cichlasoma bimaculatum*, in the range of 20–36 C, the specific dynamic action expenditure (SDA) is greater at the upper and lower temperature extremes. The SDA was found by taking the difference of O_2 consumption of fed and unfed fish. In addition, energy consumed was similar between 20 and 32 C, indicating a system similar to that located for walleye by nutritional studies alone.

The application of K_3 conversion efficiencies to nutritional studies on walleye clarifies, to some extent, energy utilization in fishes. If weekly maintenance rations (parallels Winberg's $0.3W^{0.8}$) are removed from the available energy, conversion decreases at lower temperatures (Table 3). This form of conversion probably is more realistic since temperature is known to affect metabolic expenditure (Beamish 1964; Beamish and Mookherjii 1964), and therefore decreases the truly available energy for growth. In addition, the observed effect of weight on gross and net conversion in essence is an effect of increased metabolism of the growing fishes, decreasing available energy, and not a decreased ability to grow per se.

The results of these laboratory feeding trials support observations made in 1969–70 on an unexploited population of walleye (Kelso and Ward 1972). During periods of low temperature (below 12 C), production in the wild was zero or negative. This condition was paralleled by lack of growth in fishes held at temperatures below 12 C. As well, the depressed growth of older fish may result from either depressed assimilation efficiency or increased metabolic demand. Our production system composed of younger fish (Kelso and Ward 1972) is consequently more efficient in utilizing available foods than the many other populations of walleye composed of old, and consequently slow growing, members.

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Predation by Rainbow Trout (*Salmo gairdneri*): the Influence of Hunger, Prey Density, and Prey Size[1]

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WARE, D. M. 1972. Predation by rainbow trout (*Salmo gairdneri*): the influence of hunger, prey density, and prey size. J. Fish. Res. Bd. Canada 29: 1193–1201.

The feeding behavior of rainbow trout (*Salmo gairdneri*) was investigated by exposing fish to several densities and sizes of prey over a range of four substrates of varying complexity. Within substrate treatments the rate of predation could be stimulated by increasing either prey density, prey size, or predator hunger. Between treatments, however, both the intensity of predation and total food consumption proved to be inversely related to the complexity of the substrate because a number of prey were able to find cover and thus escape detection.

The results further indicate that the nature of foraging behavior may be controlled by a critical rate of food capture. If trout did not exceed an average of .058 captures sec,$^{-1}$ substrate-oriented search proceeded to wane.

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Dans le but d'étudier leur comportement alimentaire, l'auteur a exposé des truites arc-en-ciel à des proies de différentes tailles et densités, sur une gamme de quatre substrats plus ou moins complexes. Sur un substrat donné, le taux de prédation peut être accéléré par augmentation de la densité ou de la taille des proies, ou de la faim des prédateurs. Cependant, d'un substrat à l'autre, l'intensité de prédation et la quantité totale d'aliments consommés varient en relation inverse de la complexité du substrat, un substrat complexe permettant à un certain nombre de proies de trouver un abri et d'échapper à la détection.

Les résultats suggèrent de plus que le comportement de fourragement peut être contrôlé par un taux critique de capture d'aliments. Lorsque les captures ne dépassent pas une moyenne de .058-sec,$^{-1}$ les truites abandonnent graduellement une recherche orientée sur le substrat.

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EARLY theories of feeding ecology were based upon the simplifying assumption that predation rates are proportional to the food supply. In fisheries biology such attempts have met with little success in forecasting the outcome of predation in natural systems, which is not surprising since there is general agreement that the diet of fish is more closely correlated with the physical and behavioral properties of the food species rather than their density, or biomass (Allen 1941; Grimas 1963; Ivlev 1961). These and similar observations clearly indicate that an adequate theory of predation must account for the basic mechanisms involved and their relation to major variables such as the physical properties of the food resource and the environment. At present few experimental studies have attempted to interpret the process of food selection in terms of the foraging characteristics of predators.

The present account contributes to this objective by considering the effects of prey density, prey size, environmental complexity, and hunger on the predatory behavior of rainbow trout (*Salmo gairdneri*). In what follows, a simple descriptive equation will be developed to evaluate the relative impact of these variables on the rate of predation by fish foraging for two natural food species (Amphipoda: *Crangonyx richmondensis* and *Hyalella azteca*).

Methods

GENERAL

The four rainbow trout used in this study ranged from 13.4 to 17.0 cm in length and were obtained from Marion Lake, B.C. (Efford 1967). For a period of several weeks after capture the fish were conditioned to feed in captivity by exposing each individual to 100 amphipods for 1 hr.

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When the same number of prey was consumed on four successive occasions, the initial training period was terminated.

In an attempt to control variations in predator hunger, the animals were deprived of food for 48 hr between meals. Preliminary trials indicated that this was sufficient time to clear the gut over the range in rations encountered during training and the experiments that followed.

All feeding trials were carried out in 227-liter (90 × 45 × 45 cm) glass aquaria. In both the experimental and holding tanks, the water temperature was maintained between 8 and 12 C. Although the background illumination was natural, direct light was avoided and experiments were conducted at the same time of day (1200–1400 hr P.S.T.) during the months of May–September.

The experimental treatment involved exposing trout to two different species of prey: adult *Crangonyx* ($\bar{X}$ = 8.1 mm long; 2.6 mg dry weight) and *Hyallela* ($\bar{X}$ = 5.7 mm, 1.0 mg). Experiments with each species were replicated independently over a range of four different substrates of varying complexity (Table 1). The diversity, or the amount of cover offered by the litter I and II treatments, was estimated by calculating the total surface area of the tank floor in contact with wood debris. Both substrates were composed of large pieces of debris (about 7 × 1 × 1 cm) and covered 6 and 15%, respectively, of the tank. Treatment III consisted of small pieces of litter (0.4–0.7 mm diameter) and covered the entire floor of the tank to a depth of 4 mm. A clean tank served as a control.

Table 1 summarizes the range in density examined for each food type. Since it was known from preliminary work that trout became satiated after consuming approximately 90 adult *Crangonyx* or 230 *Hyalella*, the highest food levels were selected to ensure that the predators could approach but not reach satiation during an experiment. Below this limit additional densities were chosen haphazardly.

To examine the role of prey size on predation, a single block of litter II experiments were conducted with juvenile *Crangonyx* ($\bar{X}$ = 4.6 mm, 0.5 mg). The densities employed in this case were identical to *Hyalella* because these animals were of similar size and weight.

With few exceptions, experiments within each food category were selected and replicated at random with two different fish.

The experimental procedure consisted of gathering the required number of amphipods from the sediments of Marion Lake and releasing them into an experimental tank 60 min before the predator. This was known to be adequate time for the prey to disperse and attain cover in experiments with stick debris. In the 50-min test period that followed, the number of amphipods attacked and captured, as well as the general pattern of predator movement, were recorded chronologically on an event recorder. At the end of an experiment the number of individuals removed by predation was determined by recovering the survivors.

The relation between prey size and predator reactive distance was investigated in a clean experimental tank. Replicate measurements were obtained by placing single *Crangonyx* of known size about 80 cm from a stationary fish. The latter had been held without food for 48 hr. The distance of reaction was defined as the distance between the predator and prey when the trout finally darted toward the food object, and was estimated in relation to a centimeter scale fastened to one side of the tank. For obvious reasons, observations were only recorded when the plane of attack was parallel to the scale.

Expression of Hunger

Numerous studies have dealt with the effect of food deprivation on feeding behavior. For example, Ishiwata (1968) and Brett (1971) found the ration consumed by salmonids (fed *ad libitum* after various periods of starvation) was inversely related to the amount of food remaining in the stomach from previous meals. Thus, if hunger is defined arbitrarily in terms of the propensity to feed it may be scaled accordingly by:

$$H = 1 - (V_t/V_{max}) \quad (1)$$

Table 1. A list of the experiments conducted to determine the effect of different substrates and prey densities on the feeding behavior of trout. The column figures indicate the number of replicate experiments. Italicized numbers indicate the number of experiments.

	Prey density												
	Adult *Crangonyx*					Adult *Hyalella*					Juvenile *Crangonyx*		
Substrate	20	40	70	100	200	20	40	70	100	200	40	100	200
			22					*27*				*6*	
Control	–	2	2	–	–	2	2	1	2	2	–	–	–
Litter I	1	2	2	1	–	–	2	–	2	2	–	–	–
Litter II	–	2	2	2	–	–	2	–	2	2	2	2	2
Litter III	–	2	–	2	2	–	2	–	2	2	–	–	–

where H is the level of hunger at time (t), V_{max} is the satiation ration, and V_t is the amount of food in the stomach at a specified time. In the analysis that follows, equation (1) is employed as a simple index to describe the change in the internal state of trout during feeding. From preliminary trials V_{max} was determined to be about 2 ml regardless of whether fish were fed adult *Crangonyx* or *Hyalella*.

Results

Role of Hunger and Prey density

Control experiments — The rate of fish predation was maximum at the beginning of all feeding trials and fell in a nonlinear fashion throughout the observation period. To determine the partial contribution of hunger and prey density to predation the experiments were pooled according to prey species and analyzed by multiple linear regression. For analysis the data were stratified into 30-sec intervals so the number of prey and predator hunger at time (t) could be correlated with the attack rate observed during the following period ($t + 1$). Only intervals in which trout searched continuously for food were considered. Table 2 summarizes the results and shows that there was a highly significant correlation ($P < .01$) between the rate of attack (N_a/t), prey number (D), and hunger (H), which can be described by:

$$\frac{N_a}{t} = b_1(D) + b_2(H) + K \qquad (2)$$

where b_1 and b_2 are constants.

For comparison, Fig. 1 illustrates the relative contribution of hunger and prey abundance to predation in one set of feeding trials with *Hyalella*. In this case, the curved line takes into account the depletion of prey between successive time intervals and was calculated from equation (2) by holding the hunger term constant (H = 1). Since this extrapolation removes the effect of prey density, the resulting disparity between the reference line and actual attack rate (data points) can be ascribed to the abatement of hunger. Although measurements were not made, the depression of feeding at low hunger levels appeared to be mediated through the element of handling time, for as the fish approached satiation they took progressively longer to manipulate food. It was not unusual for prey to be taken

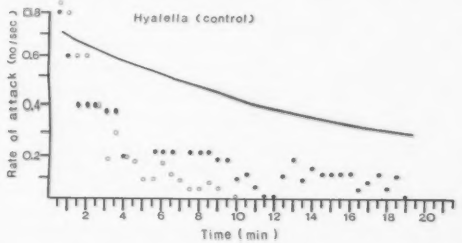

FIG. 1. Progressive decline in the rate of attack during two control experiments involving 200 *Hyalella*. Data obtained from two fish are distinguished by open and closed circles. The curved line illustrates the expected rate of attack that can be attributed to the depletion of prey between successive time intervals.

TABLE 2. Regression constants relating the attack rate during interval (t) to the hunger of the trout and the number of prey 30 sec earlier. The constant b_1 indicates the partial effect of prey density, b_2 the partial effect of hunger, and K the y intercept. The standard deviations and correlation coefficients (r_1 and r_2) are given for both constants. R_2 is the total amount of variability accounted for by regression, while n represents the number of time intervals obtained by pooling all experiments for each food category.

Prey	Experiment	n	r_1	Density (b_1)	r_2	Hunger (b_2)	K	R^2
				Control				
Hyalella	9	93	.71	0.0024±0.0002	.62	0.424±0.064	−0.21	0.66
Crangonyx	4	50	.78	0.0045±0.0005	.77	0.360±0.044	−0.13	0.83
				Litter I				
Hyalella	6	78	.62	0.0023±0.0003	.49	0.345±0.072	−0.18	0.57
Crangonyx	6	52	.89	0.0075±0.0005	.51	0.145±0.035	−0.05	0.88
				Litter II				
Hyalella	6	65	.81	0.0028±0.0002	.42	0.241±0.067	−0.19	0.69
Juvenile *Crangonyx*	6	66	.68	0.0026±0.0003	.65	0.326±0.162	−0.13	0.50
Crangonyx	6	51	.65	0.0053±0.0009	.46	0.223±0.061	−0.13	0.62

into the mouth and rejected several times before they were actually swallowed.

Litter experiments — When litter was present the rate of attack and total food consumption by trout proved to be inversely related to the complexity of the experimental substrates. Such a result can be attributed largely to the element of prey exposure since a substantial segment of both food species sought cover beneath debris and thereby escaped detection. In contrast, there was no overt change in predator behavior; they continued to respond visually to prey and maintained the same vertical searching position (roughly 10 cm above the substrates) as they had in the control situation.

Figures 2 and 3 illustrate that the relation between prey density and the number captured in each treatment can be described by a family of straight lines which pass through the origin. In other words, irrespective of their density a constant proportion of prey proved to be exposed and vulnerable. The degree of exposure, however, was dependent upon the complexity of the substrates, as significantly fewer amphipods were cropped in litters II and III (Table 3). Although this inverse trend clearly held for both species, their respective risks to predation were by no means identical. Significantly fewer *Crangonyx* (43% of the populations relative to 66% for *Hyalella; P* < .05) were captured in litter II, while the converse was true in litter III (*Crangonyx* = 31%, *Hyalella* = 16%; *P* < .05). For the previous comparison, the litter II experiments with adult and juvenile *Crangonyx* were pooled, as there was no significant difference in the proportion of animals removed by fish under comparable conditions.

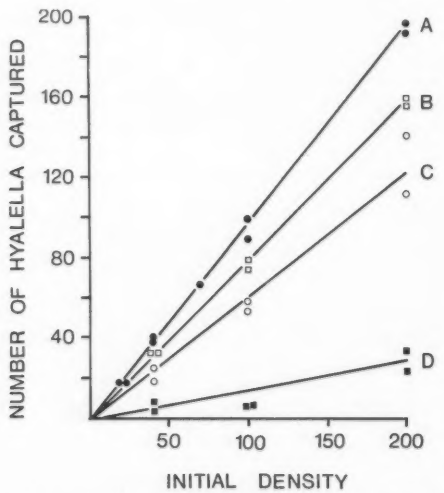

FIG. 2. Relation between substrate diversity and the number of *Hyalella* captured after 50-min exposure to trout. A, control; B, litter I; C, litter II, D, litter III.

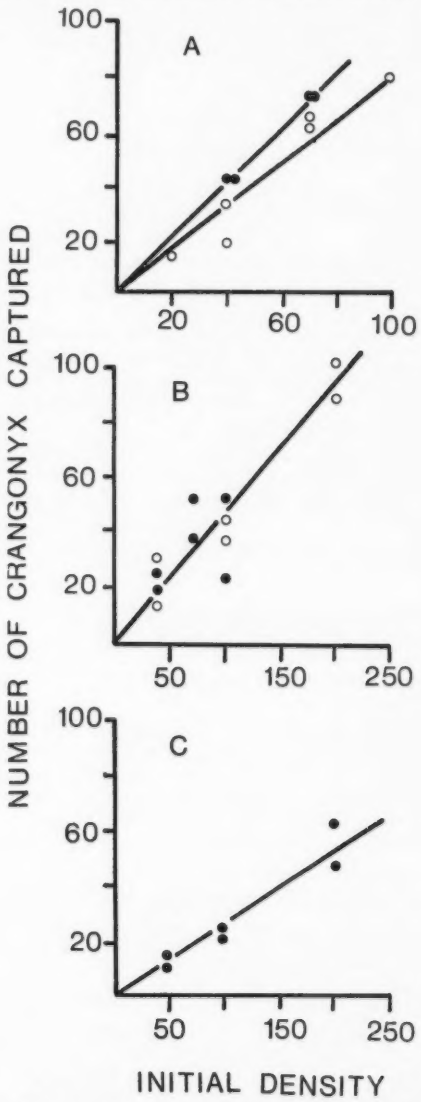

FIG. 3. Relation between substrate diversity and the number of *Crangonyx* captured after 50-min exposure to trout. A, closed circles represent the control; the open circles, litter I; B, litter II, the closed circles indicate experiments conducted with adult *Crangonyx;* the open circles, juvenile *Crangonyx;* C, litter III.

TABLE 3. Comparison of the proportion of *Crangonyx* and *Hyalella* captured in each substrate treatment. *n* indicates the number of experiments, *r* is the correlation coefficient relating the number of prey captured to their initial density, and PE signifies the proportion of animals captured (slope). V_b is the variance of the slope. Treatment slopes not bracketed by the same vertical line are significantly different at the .05 level or less.

Substrate Treatment	*n*	*r*	V_b	PE
		Hyalella		
Control	9	0.99	.0010	0.98
Litter I	6	0.99	.0003	0.79
Litter II	6	0.98	.0050	0.66
Litter III	6	0.90	.0014	0.16
		Crangonyx		
Control	4	1.00	.0000	1.00
Litter I	6	0.97	.0133	0.90
Litter II	12	0.91	.0040	0.43
Litter III	6	0.97	.0016	0.31

Since trout did not respond to concealed animals, the available density of food in the litter experiments can be defined as the number of individuals exposed in the water column and on the substrate surface at the beginning of each time interval (t). By adopting this convention it is possible to determine if the performance of trout was directly impaired by the presence of litter. If there was no overt effect, then multiple regression analysis should produce a set of constants (b_1, b_2, and K) similar to the controls. Unfortunately, the litter III results could not be analyzed in detail because it was impossible in many cases to accurately discriminate between attacks directed toward prey and pieces of litter resembling food.

Regression analysis verified that the values of b_1 and K were not significantly affected by the experimental substrates, except when adult *Crangonyx* appeared in association with litter I (Table 2). In this instance, prey density (as reflected by b_1) exerted a greater influence on predation than in any other treatment. On the other hand, there was a progressive decline in the magnitude of b_2 throughout the litter series. Though this result implies a parallel change in some behavioral component, the effect of this alteration proved to be relatively small. Hence, I assumed an increase in the diversity of the physical surroundings mainly operated to lower the number of exposed prey (D_e). Equation 2 can be expanded to handle this aspect accordingly:

$$\frac{N_a}{t} = b_1(D_e) + b_2(H) + K. \qquad (3)$$

ROLE OF PREY SIZE

Regardless of the substrates involved, adult *Crangonyx* were attacked about twice as fast as the alternative prey for any given combination of food density and predator hunger (Table 2). To a large part this result can be attributed to the behavior of the predator, for there was a strong positive correlation (r = .98) between the size of individual prey and the distance from which they were approached (reactive distance) by fish:

Prey length (*mm*)	*n*	Mean reactive distance (*cm*)
4	9	18±3
5	17	22±2
6	9	28±5
7	21	28±3
9	28	35±3

where (n) indicates the number of observations obtained from two fish; the mean and 95% confidence limits are expressed to the nearest significant figure.

There is little doubt this property of foraging behavior will contribute to, if not explain, the relation between prey size and predation rate because an increase in reactive distance will accelerate the rate of discovery.

PREY CAPTURE SUCCESS

Rainbow trout were highly successful in capturing all sizes of prey offered and did not appear to be hampered to any degree by the experimental substrates. The efficiency of prey capture was calculated as the ratio between the number of prey ingested to the number attacked, and was found to range from a high of 91 ± 10% in the controls to a low of 84 ± 16% in litter II (mean ± 95% confidence limit). Since these differences are not significant, nor was there any effect of prey size, the rate of food intake (N_c/t) can be described for all experimental treatments by treating capture success C as a constant ($\overline{X}$ = .87) and coupling it with (3):

$$\frac{N_c}{t} = [b_1(D_e) + b_2(H) + K]\,C. \qquad (4)$$

Equation (4) summarizes the contribution of various elements to feeding intensity, but must be qualified since it tacitly implies there is no limit to the rate of predation. Such an assumption is unreasonable and is actually refuted by the data (Fig. 4). If we compare the attack rate during the first 30 sec (H = 1) of the control, litter I, and litter II experiments in relation to the number of exposed

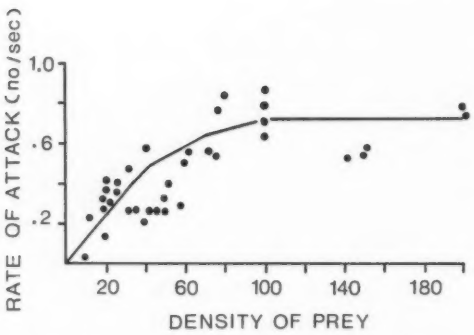

FIG. 4. Functional response of trout to prey density. The rate of attack was determined over a 30-sec time period. In each case predator hunger was maximal with respect to equation (1). The curve was fitted by eye.

prey, it is clear that trout achieved an average maximum performance of .67 attacks sec^{-1} at high food levels. Consequently, the explicit restriction of (4) is that N_c/t cannot surpass this limit regardless of the abundance or size of the food particles, or predator hunger.

The second point reflected in Fig. 4 is the nonlinearity of the functional response of trained fish to prey density. This phenomenon is well established and has been shown to result from the act of handling food (Holling 1966; Rashevsky 1959). The amount of time predators require to attack and swallow individual prey is given by the reciprocal of the maximum feeding rate, and for trout is equivalent to an average of 1.5 sec. Although it was mentioned that the duration of this interval appeared to be related to predator appetite, the form of the functional response over all food and hunger levels can be approximated by (4) by imposing an upper limit on the attack rate.

PREDATOR MOVEMENT

There were two distinct phases of predator movement during the feeding trials (Fig. 5). The first (CSP) was initiated immediately after the fish were released and was characterized by a period of intensive foraging. Throughout this interval trout adopted a vertical searching position some 10 cm above the substrate between attacks, and oriented downward at a 10–20 degree angle to face the floor of the tank. In general, the duration of the primary search phase proved to be dependent on the size and concentration of exposed prey (Fig. 6). The second phase of predator movement (ISP) was initiated by a sudden shift in attention from the substrate to one of apparent undirected swimming activity. During

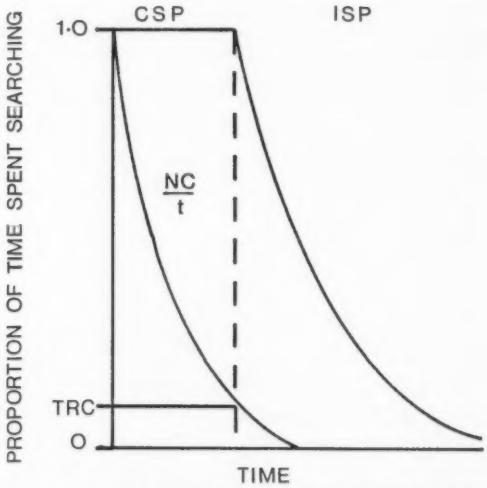

FIG. 5. A schematic illustration of the general nature of search behavior. CSP represents the interval in which fish devoted complete attention to foraging for amphipods. During the second phase (ISP) the initial search pattern waned. The curve N_c/t represents the progressive decline in the capture rate throughout the experiments, and TRC indicates the 'threshold' rate of capture associated with the shift in behavior.

this interval the fish spent proportionally less time in their characteristic searching posture and made frequent trips between the tank floor and water surface or generally wandered about the tank.

To explain this abrupt change in behavior it was postulated that trout may have to exceed a threshold rate of capture (TRC) before they will persist with a particular hunting pattern. If this conjecture is valid, then we would expect the rate of predation, prior to the termination of the primary search phase, to be constant irrespective of the predator's state of hunger or the food species involved. Linear regression verified that the value of TRC ($\overline{X} = 0.58 \pm$ 95% confidence limit of .017) was independent of hunger ($n = 19$; $r = .11$) when trout were exposed to *Hyalella* but not *Crangonyx*. In the latter case the correlation was significant ($n = 17$; $r = .44$): TRC = .054 (H) + .025. Although this development fails to support the contention of a constant threshold, both sets of data unequivocally demonstrated that substrate-oriented search proceeded to wane once the rate of capture fell within a range of .02–.10 captures $sec.^{-1}$ Thus, for the purposes of this paper it is assumed that the search 'threshold' can be represented by an average value of .058 captures $sec,^{-1}$ regardless of food density or predator hunger.

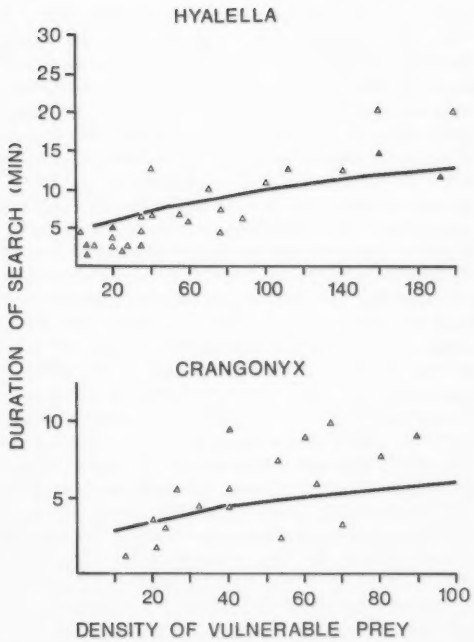

FIG. 6. Comparison of the expected duration (curve) of the initial search phase (CSP) and the observed time (data points) in experiments with *Hyalella* and adult *Crangonyx*. The curves in each case were obtained by solving equation (4).

As a final check of its descriptive power, equation (4) was used to calculate the expected duration of the initial search phase over the range in food densities employed. To simplify analysis, the experiments were arranged into two blocks according to prey size: adult *Crangonyx;* and *Hyalella* and juvenile *Crangonyx*. Appropriate parameter values were calculated for each food category by averaging the constants listed in Table 2 over all substrate levels. The equation was then solved iteratively to determine the time trout were expected to take to reduce selected densities of prey down to a level which would produce a feeding rate equivalent to the search threshold (.058 captures sec^{-1}). Figure 6 illustrates the result of this exercise and would appear to support the conclusion that (4) provides a satisfactory description of the observations.

Discussion

The foraging behavior of trout clearly reinforces the tenet that the intensity of predation by fish is not proportional to their food supply. There are several reasons for this phenomenon, one of which is the element of handling time. Holling (1966) and Rashevsky (1959) discussed the role of this component and concluded on empirical grounds that the rate of food consumption must eventually reach an asymptote because predators are obliged to devote some time to handling each food particle they attack. The functional response of trout (Fig. 4) is in complete agreement with the model they propose.

Prey exposure is an additional variable that is seldom dealt with in laboratory studies but is likely to play a major role in natural systems, since my observations imply trout are obligate visual predators. Various accounts support such a conclusion and indicate it may pertain to other salmonids as well. Johannes and Larkin (1961), for example, found rainbow trout foraged for *Gammarus* by remaining some distance from areas of vegetation and would only move in to attack individuals that appeared at the periphery of weed beds. Brook trout (*Salvelinus fontinalis*) may utilize similar tactics, since Smith (1961) noted the population in Crecy Lake did not benefit appreciably from the increase in benthic production that followed a program of artificial fertilization. A considerable portion of the resulting production was made up of small infaunal groups such as oligochates and amphipods that were seldom taken by fish either before or after nutrient addition. Related work by Macan (1966) for brown trout (*Salmo trutta*) and Schutz (MS 1969) for cutthroat (*Salmo clarki*) supply further evidence that exposure may often be a necessary condition for prey detection.

The apparent dependence of feeding rates on food size is well known and is a third general characteristic of fish predation exhibited by trout. In the case of visual predators, Hester (1968) demonstrated from the principles of visual mechanics that large, conspicuous objects stand a high risk of discovery. The correspondence between prey size and predator reactive distance clearly verifies this conclusion and indicates a proximate mechanism to account for the phenomenon of size-selective predation. Brawn (1969) reported a similar response for cod (*Gadus morhua*). For these and related reasons, numerous individuals (e.g. Allen 1941; Beukema 1968; Royama 1970) have stressed that a biologically meaningful definition of prey availability (or risk) must take into account the physical features of prey, as well as their dispersion and numerical abundance.

From a different point of view, predation is also governed by the internal nutritional state of predators. Accordingly, the description of hunger employed above was extremely simple, yet proved to be highly correlated with feeding intensity. At present the mechanisms underlying the regulation of food intake are not fully understood (Teitelbaum 1966),

but there seems to be general agreement that stretch receptors in the gut musculature provide an immediate signal to the central nervous system to control feeding (Hamilton 1965). This pathway has been indirectly confirmed for both vertebrates and invertebrates, since feeding rates in both cases tend to be inversely related to stomach fullness. Though this generalization may prove useful, it should only be regarded as a provisional index of hunger because Beukema (1968) cautioned that such expressions do not account for alterations in feeding behavior after long periods of food deprivation.

Within the physical constraints of the experimental system there was a strong indication that predator movement was associated with the rate of food capture. In this connection it is not surprising to find that many animals will alter their search pattern and increase the frequency of turning to maintain themselves in close proximity to food aggregations (Beukema 1968; Croze 1970). If the response of trout can be viewed as a rough experimental analogue, the results allow the conjecture that the rate of capture may serve as an internal signal to control foraging behavior.

To clarify this hypothesis I have summarized the contribution of the elements of predation covered above in the form of a control diagram (Fig. 7). For the purpose of discussion, the position of a predator in space can be defined by a set of coordinates (X and Y) if we assume that it maintains a constant angle of orientation with respect to some arbitrary reference point. As we have shown, the spatial component in conjunction with the predator's state of hunger and the density of prey within the perceptual field participate in the attack process to generate an instantaneous rate of capture. This signal in turn is presumed to be transformed through a neurological pathway into a 'decision-making' variable (S). If the resulting difference or error (E) between the search threshold (TRC) and (S) is negative, the model predicts that the prevailing foraging pattern will be maintained. In addition, as a secondary response the magnitude of the error could accelerate the incidence of turning and thus hold the animal within a restricted area. On the other hand, a positive error signifies a low rate of 'reward' (food intake) in which case the predator is expected to either modify its search behavior or leave the area in question. It should be pointed out that the model allows for the provision that the search threshold may be hunger-dependent, but this is not a central issue.

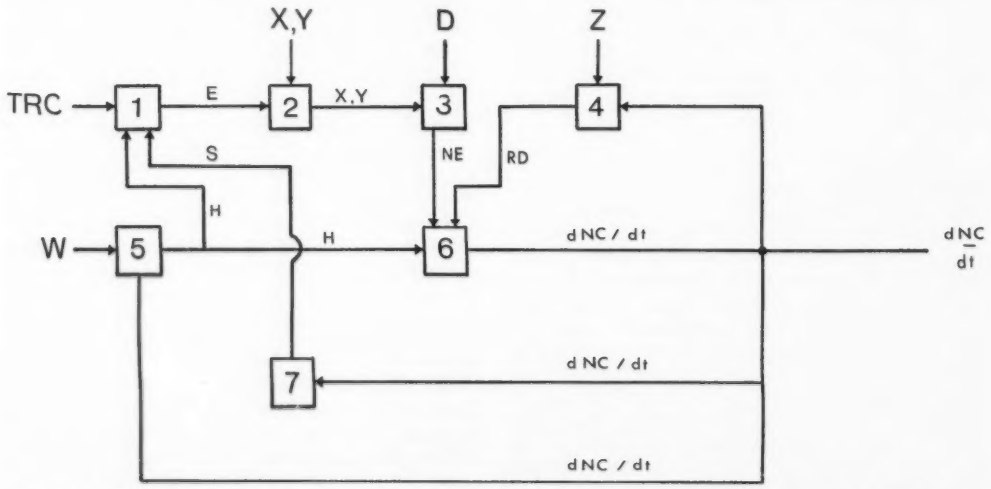

FIG. 7. A model to account for the movement of trout during feeding. The components designated by large capital letters represent a set of initial conditions; the boxes, subsidiary processes, and the arrows represent input–output variables. Initial conditions at time (t): X, Y, vertical and horizontal coordinates of predator; W, volume of food in stomach; D, density of prey; Z, state of experience of predator with specific prey; TRC, threshold rate of capture.

Subsidiary process: 1, comparative process: determines the error (E) between the reference value (TRC) and the signal of prey capture (S); 2, decision making process: if (E) is positive the search mode is altered; 3, prey vulnerability process: determines the array of prey within the perceptual field (NE); 4, learning process: alters the reactive distance (RD) of the predator to specific prey; 5, hunger process: changes the state of hunger (H); 6, attack process: generates the instantaneous rate of food capture (dNC/dt); 7, neurological process: transforms the instantaneous rate of prey capture into an input signal (S).

At present the validity of such a scheme cannot be confirmed, though there is some indirect support for it in two recent papers dealing with predation by birds. Royama (1970) postulated that the movements and selection of food by titmice (*Parus major*) may be directed to maximize their hunting efficiency, or what he called the 'profitability' of foraging. By analyzing his argument it is clear that he tacitly assumes the existence of a central process that assesses the 'reward' to be gained by searching in selected sectors of the hunting range. Consequently, the model I propose not only complements his theory but would seem to form a sufficient condition for its operation. In a somewhat related study, Croze (1970) utilized 'give-up' time as a measure of the persistence of carrion crows (*Corvus corone*) to continue foraging in a restricted area. He defined this parameter as the time the birds passed in the experimental zone between finding the last piece of food and flying away, and showed that it was independant of food concentration. Considering that the reciprocal of this interval is equivalent to the concept of a search threshold, our approaches are completely congruent.

One final characteristic worthy of note concerns the contribution of associative learning to predation. This aspect was not investigated in the present study but was added to the feeding model (process 4), because Beukema (1968) and Ware (1971) found that fish can elevate their reactive distance if they acquire sufficient experience with particular prey. Figure 7 points out the adaptive significance of this pathway by illustrating that it acts as a positive feedback to improve the efficiency of predation.

On the basis of the foregoing considerations there seems to be adequate evidence to demonstrate that predation rates are determined largely by the interaction between the state of hunger and experience of fish, as well as their movements in relation to the available food supply. We must recognize, however, that while these conclusions may hold for experimental systems we have yet to develop a general theory of predation that can be applied with confidence to natural systems.

Acknowledgments

I am grateful to Dr I. Efford for his encouragement and financial support during this study. Many members of the Marion Lake IBP group provided valuable contributions, but I would especially like to acknowledge the help of P. Pearlstone and R. Delury. Drs C. S. Holling, L. Dickie, and S. Kerr offered helpful discussion and criticism of an earlier draft of the manuscript.

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Biodegradation of Trisodium Nitrilotriacetate in a Model Aerated Sewage Lagoon

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RUDD, J. W. M. AND R. D. HAMILTON. 1972. Biodegradation of trisodium nitrilotriacetate in a model aerated sewage lagoon. J. Fish. Res. Bd. Canada 29: 1203–1208.

The effect of temperature on the biodegradation of trisodium nitrilotriacetate (Na_3NTA) in a model aerated sewage lagoon was investigated. The percent breakdown of incoming NA_3 NTA at 15, 5, and 0.5 C was 93, 47, and 22% respectively. On the basis of these results, it can be expected that aerated sewage lagoons operating in the "mid-continental" climate of the Canadian prairie provinces would discharge at least 50% of incoming Na_3NTA for 6 months of the year.

The presence of a bacterial predator, *Daphnia*, was found to enhance the breakdown of Na_3NTA.

Fluctuations in Na_3NTA removal appeared to increase with decreasing model lagoon temperature.

RUDD. J. W. M. AND R. D. HAMILTON. 1972. Biodegradation of trisodium nitrilotriacetate in a model aerated sewage lagoon. J. Fish. Res. Bd. Canada 29: 1203–1208.

Nous avons étudié l'influence de la température sur la biodégradation du trisodium nitrilotriacétate (Na_3NTA) dans un étang modèle d'aération des eaux d'égoût. Les pourcentages de décomposition du Na_3NTA d'arrivée à 15 C, 5 C et 0.5 C sont de 93%, 47% et 22% respectivement. D'après ces résultats, on peut s'attendre que les étangs d'aération des eaux d'égoûts opérant dans les conditions "mi-continentales" des provinces des Prairies canadiennes déchargent au moins 50 % du Na_3NTA d'arrivée durant six mois de l'année.

La présence du bactériophage, *Daphnia*, accentue la décomposition du Na_3NTA.

L'élimination du Na_3NTA semble varier davantage lorsque la température de l'étang baisse.

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IT has been estimated that some 60% of the phosphorus reaching lakes originates as detergent polyphosphates (Vollenweider 1968). Phosphorus is generally conceded to be the most controllable element which limits the growth of aquatic plants. In view of the accelerated eutrophication which is so obvious in lakes today great emphasis is being placed upon the control of phosphorus and, in particular, upon the elimination of phosphates from detergents (Likens 1972).

The trisodium salt of nitrilotriacetic acid (NTA) has emerged as the most acceptable replacement for phosphate as a builder in detergents. NTA does not appear to occur widely in the environment and, therefore, may be classified as a novel compound. Being novel, questions were raised regarding its effect upon human health and the environment.

Short of inhalation or actual ingestion of laundry products the most obvious source of NTA intake for humans would be via water supplies. Environmental concerns would also appear to be centered upon aquatic systems. All investigations of these areas of concern should be based upon a knowledge of the concentration of NTA to be expected to occur in water. Most of the NTA used in detergents could be expected to be exposed to some type of sewage treatment system so the effect of NTA loading has been investigated in a number of systems (Swisher et al. 1967; Shumate et al. 1970; Thompson and Duthie 1968), as has the efficiency with which

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such systems degrade the compound (Bouveng et al. 1970; Gudernatsch 1970; Shumate et al. 1970; Swisher et al. 1967; Thompson and Duthie 1968). Efficiency of degradation appears to depend upon many factors, one of the most important being temperature.

Sewage lagoons are used widely in Canada. In the prairie provinces some 198 communities are serviced by such facilities. Such lagoons may be aerated or unaerated and are operated at the very low ambient temperatures typical of an area which is technically described as being "mid-continental." The mean daily temperature of this area is below O C for 5 months of the year (Anon. 1968) and one study has shown that a major lagoon in the area operated at a temperature of less than 1 C for 3 months of the year (Anon. 1969). It therefore appeared that, should NTA become widely used in Canada, such lagoon sewage treatment systems might be a major source of NTA input to the aquatic ecosystem.

A study involving an actual system was thought to be impractical and indeed, ill-advised should NTA not be degraded or should it affect operation of the system. Therefore, a model system was designed, constructed, tested for its ability to reflect the operating characteristics of an actual lagoon, and then tested for its response to NTA loading.

Materials and Methods

A part of the Charleswood Lagoon in the City of Winnipeg was designed to enable an assessment of three different aeration systems. The operating characteristics of these systems were the subject of a year-long study (Anon. 1969) and they are still under continual monitoring. In order to take advantage of these background data our model was designed to imitate the Charleswood Lagoon and, in particular, its Air-Aqua® system.

The Charleswood Air-Aqua system was composed of primary and secondary cells with a total volume of 15 $\times$ 10^6 imperial gallons. Aeration was supplied by polyethylene tubing laid across the bottom of the cells. Air bubbles escaped from openings in the upper surface of this tubing. The aeration system was tapered so that the interval between aeration tubes was smallest at the influent end of the primary cell where the BOD_5 was highest.

The model lagoon has been described in detail elsewhere (Rudd MS 1972). It consisted of primary and secondary cells, each with a capacity of 269 liters. The cells were temperature controlled and aerated in a manner physically similar to the actual lagoon. Raw sewage was collected from the main control chamber of the Charleswood Lagoon, frozen, and stored until needed. Enough sewage for 1 week's operation of the model was collected at each time. Raw sewage was fed to the model at a rate which produced the 30-day retention time attributed to the actual lagoon (Anon. 1969).

In order to establish the basic operating characteristics of the model and to enable comparison with the Air-Aqua system the following analyses were performed: BOD_5, COD, total suspended solids, total dissolved phosphate, ammonia, nitrite, nitrate, and *p*H (Anon. 1965). The oxygen tension of both cells was monitored and adjusted as necessary to produce a minimum dissolved oxygen concentration of 2 mg O_2/liter.

NTA was added to the raw feed sewage at a concentration of 20 mg $NTA \cdot 3Na \cdot H_2O$/liter (equivalent to 14.0 mg NTA/liter). The effect of this addition on the lagoon operation was monitored by observing the parameters listed above and by following the NTA concentration in the effluent of the secondary cell. NTA analyses were done by the gas-chromatographic method of Murray and Povoledo (1971) following sample pretreatment by centrifugation at 9000 rpm for 20 min, filtration through an 0.45μ pore-size membrane filter, acidification to *p*H 2.5, and freezing.

Results

Some operating characteristics of the model, before introduction of NTA, are compared with data from the Charleswood system in Table 1. The model studies were done during a 20-day period at 5 C which is a transitional temperature for the actual lagoon, being observed for 1–2 months in spring and fall. The data from the Charleswood system therefore represents its characteristics during a period of maximum instability.

To ensure that the model was a fair reflection of the Air-Aqua system as well as to detect any changes which might be attributable to NTA such observations were continued at other temperatures and during NTA loading. These data are presented in Table 2. The average values obtained from the Air-Aqua system for the winter of 1970–71 were determined during the period in which the model lagoon was operating (Carrol 1971). However, the model

TABLE 1. Comparison of the operational characteristics of the model lagoon and the Charleswood Air-Aqua system during a 20-day test period prior to NTA loading.

Test	Air-Aqua	Model Lagoon
DO	6.0 mg/liter	11.5 mg/liter
Influent BOD	240 mg/liter	240 mg/liter
Effluent BOD	15.8 mg/liter	20 mg/liter
BOD_5 removal efficiency	93%	92%
Effluent suspended solids	21 mg/liter	10 mg/liter
Suspended solids removal efficiency	85%	96%
Temperature	~5C	5C

TABLE 2. Comparison of the operational characteristics of the model lagoon and the Charleswood Air-Aqua system after NTA loading of the model.

Test	Air-Aqua		Model Lagoon
Effluent *p*H	7.7[a]		7.8
Effluent total phosphate	30.5 mg-PO_4.liter[a]		23.1 mg-PO_4/liter
Effluent inorganic nitrogen	24.0 mg-N/liter[a]		27.0 mg-N/liter
BOD_5 removal efficiency	85%[b]	80%[a]	91%
Effluent COD	–		86.1 mg-COD/liter
Suspended solids removal efficiency	93%[b]	82%[a]	98%

[a]Values obtained from January 1968 to September 1969.
[b]Values obtained from November 1970 to April 1971.

lagoon operated at summer and winter temperatures during this period. Therefore, average Air-Aqua values obtained from January 1968 to September 1969 are also presented (Anon. 1969).

BIODEGRADATION OF NTA

The concentration of NTA in the secondary effluent of the model lagoon at 15 C, 5 C, and 0.5 C are shown in Fig. 1. The average values of NTA concentrations in the secondary effluent at these temperatures were found to be 1.0, 7.7, and 10.9 mg NTA/liter, respectively. Perturbations in effluent NTA concentrations were observed at all temperatures (but expecially at 5 C and 0.5 C). In the calculation of average effluent NTA concentrations these fluctuations were taken into account since this phenomenon may also occur in the actual lagoon. Therefore, the 15 C, 5 C, and 0.5 C averages include values between days 148–165, 103–128, and 43–92, respectively. Since 14.0 mg NTA/liter was added to the raw sewage influent, the average percent breakdown at 15 C, 5 C, and 0.5 C was 93%, 45%, and 22%, respectively. These data do not include any NTA which might have been present in the raw sewage. It was not possible to analyze for NTA in raw sewage because of unidentified components which interferred with the gas chromatography. Considering the low level of usage of NTA based detergents it is unlikely that raw sewage would be a significant source during this period of time.

The average rates of NTA breakdown as a function of temperature are shown in Fig. 2. At 15 C, 5 C, and 0.5 C these rates were 0.45, 0.22, and 0.12 mg NTA/liter day, respectively. The average breakdown rates were calculated by subtracting the average effluent NTA concentrations from the constant influent NTA concentration (14.0 mg/liter). These differences were then divided by the model lagoon retention time (30 days).

The initial increase in effluent NTA concentration as well as the increase following the temperature shift from 5 C to 0.5 C likely reflected hydraulic mixing in the system (Fig. 1). It is therefore difficult to determine the length of acclimation period in these

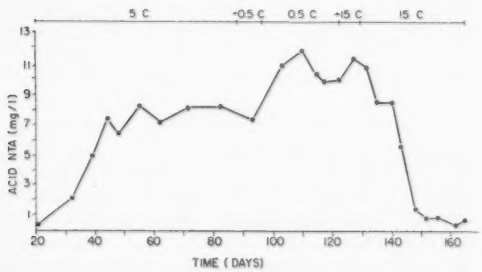

FIG. 1 Concentration of NTA in the secondary effluent of the model lagoon.

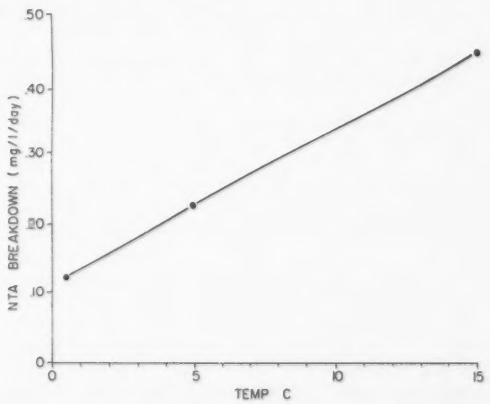

FIG. 2 Rate of NTA breakdown, by the model lagoon, as a function of temperature.

instances. However, as in other investigations (Bouveng et al. 1968; Forsberg and Lindqvist 1967; Pfeil and Lee 1968; Shumate et al. 1970; Swisher et al. 1967; Thompson and Duthie 1968) a period of acclimation can be demonstrated, in this case after the temperature shift from 0.5 C to 15 C. Warming of the lagoon began on day 122. However, it was not until 21 days later that the effluent NTA concentration began to drop rapidly. In this case the 21 days is believed to approximate the time required for a population shift in favour of those organisms capable of degrading NTA at mesophilic temperatures. It is believed that this is a valid approximation of the acclimation period because in this instance the NTA concentration in the lagoon before acclimation, was approximately the same as the influent concentration. This minimizes the effect of hydraulic mixing.

Raising the temperature of the model to 15 C resulted in an algal bloom followed by a bloom of *Daphnia*. During this period the standard estimate of suspended solids showed two large fluctuations. It ceased to be a valid estimate of the efficiency of the model in that these fluctuations, almost certainly, were due to algal and crustacean biomass (Fig. 3). While it was not possible to separate the algal cells from suspended solids, the *Daphnia* were of sufficient size to permit us to effect a clean separation. Thus it can be shown (Fig. 3) that classical suspended solids actually decreased during the *Daphnia* bloom. Suspended solids may be taken as a reflection of bacteria cells present in the water column. It is therefore of interest to note that while suspended solids decreased, due to grazing by *Daphnia*, the level of NTA degradation remained high. This observation is consistent with those of other workers (Javornicky and Prokesova 1963; Straskrabova and Legner 1969) where it has been noted that predation can enhance the physiological state of a bacterial population and, therefore, its ability to effectively utilize a substrate. Attempts to evaluate rates of bacterial biodegradation, in sewage or other mixed systems, which neglect nonbacterial members of the community must, therefore, be interpreted with caution.

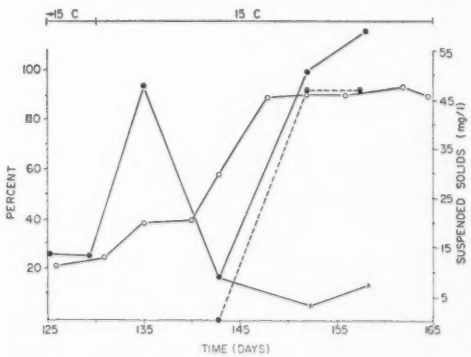

FIG. 3 Total suspended solids in the secondary effluent of the model lagoon, solid circles; suspended solids excluding *Daphnia*, crosses. Percent breakdown of NTA, open circles; percent suspended solids composed of *Daphnia*, broken line.

Discussion

It has been shown that a model lagoon can be constructed and operated in a manner which results in a good, if somewhat more efficient, imitation of an actual aerated lagoon. The model may be somewhat more efficient than the actual lagoon because it did not have to contend with diurnal load and flow fluctuations. Inasmuch as the standard chemical analyses which are applied to operating lagoons indicate a successful mimicry it is reasonable to suspect that the behaviour of a model with regard to NTA loading is also a reasonable approximation of the behaviour of a full-scale lagoon. Therefore, we may conclude that the results indicate NTA loading is unlikely to affect the normal operation and efficiency of a lagoon sewage treatment system.

Shumate et al. (1970) have found that at constant NTA feed levels, NTA removal in an activated sludge plant fluctuated around a mean value. Possibly this could have occurred because of fluctuation in the population of the sewage treatment system. The population fluctuations could have resulted from variation in flow, sewage concentration, or sewage components. Thus, if the population is constantly shifting, there will be a need for continuing acclimation of the population for the utilization of minor sewage components (such as NTA). If the acclimation rate is slower than the rate of population shift, it would be expected that the NTA removal would fluctuate about a mean value. For the case described above the variability of NTA removal increased with increasing NTA dosage. This phenomenon was also observed in the model lagoon (Fig. 1) which was fed NTA at a constant level of 14.0 mg NTA/liter. In this case fluctuations appeared to be more pronounced at 5 C and 0.5 C than at 15 C (Fig. 1). These fluctuations are significantly larger than the standard deviation of the chromatographic analysis. The greater fluctuation of NTA breakdown at psychrophilic temperatures may occur as a result of slower acclimation rates at lower temperatures while at 15 C the rate of acclimation may be rapid enough to reduce the

fluctuation in NTA breakdown during population shifts.

In view of these perturbations of an otherwise stable system and in view of the dependance of basic NTA degradation efficiencies upon temperature we may conclude that the operating temperatures of lagoons will have profound effects upon their ability to degrade NTA.

If the 1968 study of the Charleswood lagoon can be considered representative of lagoon operation in the "mid-continental" region, operating temperatures below 10 C for 7 months of the year would be typical. For 3 months of this period operating temperatures would range between 5 C and 1 C resulting in NTA degradation processes of less than 50% efficiency. For an additional 3 months the temperatures would be less than 1 C resulting in efficiencies below 15%. However, it should be recognized that, at present, many lagoons in Canada are not aerated, in which case the lagoons will be ice-covered for about 6 months. Ice cover results in prompt attainment of anaerobic conditions which, on the basis of other studies (Krumrei 1970) would suggest that no degradation of NTA will occur during this period of time. It is concluded that sewage lagoons in this climate, if discharging during winter months, can be expected to discharge between 50% and 100% of the incoming NTA.

Since suspended solids and BOD removal efficiencies remained high (>90%) during the period the model operated at psychrophilic temperatures, the reduced efficiencies of NTA removal also suggest that NTA does not adsorb to solids in the lagoon and therefore does not settle out.

The significance of this discharge would depend upon dilution by receiving waters and upon the ability of the biota of said water to degrade the incoming NTA. Thus it would be prudent to initiate further research, at winter temperatures, designed to test the effects and fate of NTA in water ways which receive raw or lagoon treated sewage. It may be that simple monitoring of the NTA levels of the receiving waters near prairie cities would provide adequate information and such a program has been instituted recently in Canada.

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NOTES

Gonad Maturation of Brook Trout (*Salvelinus fontinalis*) in a High Mountain Lake Under a Modified Photoperiod

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LARSON, G. L. 1972. Gonad maturation of brook trout (*Salvelinus fontinalis*) in a high mountain lake under a modified photoperiod. J. Fish. Res. Bd. Canada 29: 1209–1211.

Gonad maturation of brook trout (*Salvelinus fontinalis*) in Pratt Lake appeared similar to those reported for other areas although the day-length cycle was greatly modified by snow cover that capped the lake until early summer.

LARSON, G. L. 1972. Gonad maturation of brook trout (*Salvelinus fontinalis*) in a high mountain lake under a modified photoperiod. J. Fish. Res. Bd. Canada 29: 1209–1211.

Les gonades de l'omble de fontaine (*Salvelinus fontinalis*) ont semblé se développer au même rythme dans le lac Pratt que dans les autres régions, en dépit de la couche de neige qui a recouvert le lac jusqu'au début de l'été et qui a modifié considérablement la durée quotidienne de luminosité.

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THIS note describes the gonad maturation in a population of brook trout exposed to an unusual natural day-length cycle. In the northern portion of its range, brook trout normally attain mature gonads in early fall (White 1934). Artifical changes of the annual day-length cycle (photoperiod), however, have increased or retarded the rate of gonad maturation of brook trout (e.g. Hoover and Hubard 1937; Hazard and Eddy 1951; Allison 1951). Henderson (1963) presented experimental evidence that the natural increase and decrease in day-length may regulate the gonad maturation cycle of brook trout.

The main question asked in this investigation was whether normal gonad maturation occurred in a high mountain lake population of brook trout presumably adapted to a photoperiod regime different from that discussed by Henderson.

The annual day-length cycle of fish in the high mountain lakes of the Western Washington Cascade Mountains is modified by an unusual winter lake cover composed entirely of a floating snow pack that may be several meters in thickness. Little light appears to penetrate into the lake until the lake cover decreases to about 1 m in thickness in early summer (Larson MS 1969). Thus, fish are exposed to near darkness during winter. After the lake cover disappears (usually between June and August) the fish are exposed to a long photoperiod, which may be maximal or even decreasing in duration (Fig. 1). Water temperatures remain low until the lake cover disappears, and then rise rapidly (Larson MS 1969).

Lake and fish — Pratt Lake, in the Western Washington Cascade Mountains, lies at an elevation of 1026 m and has a maximum surface area of 17.6 ha (Wolcott 1961). Brook trout were planted in the lake in 1934 (Washington State Department of Fisheries personal communication).

In 1964, gonads were obtained from 45 brook trout collected on July 19, August 9 and 16, and September 13 by angling. All collections were preserved in 10% formalin. One month after preservation the fish were weighed and measured. Fish were weighed to the nearest 0.1 g and the gonads to the nearest 0.01 g; excess fluid was blotted before weighing. Ages were determined by scale analysis.

The fish averaged 69.8 g in weight, ranging from 38.2 to 172.1 g. The average standard length was 15.7 cm, the range being from 12.5 to 18.3 cm. The ages averaged 2.5 years and ranged from 2 to 5 years.

Photoperiod and temperature — Day lengths were calculated from a tide book giving times for sunrise and sunset at 48° N.

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The thickness of the lake cover was estimated by plotting terrestrial snow depth versus depth of the lake cover on Mowich Lake, a similar lake nearby, in 1967 (Larson MS 1969), and making use of data on terrestrial snow depth near Pratt Lake in 1964.

The maximum estimated thickness of the lake cover on Pratt Lake was about 2.8 m, in March (Fig. 1). The lake cover was about .5 m in thickness on June 12, completely disappearing by July 19. On the basis of these changes in thickness, the annual photoperiod of fish in lake was estimated as shown in Fig. 1.

During winter water temperature was assumed to be approximately 4 C, as at nearby Mowich Lake (Larson MS 1969). After the lake cover disappeared surface water temperatures rose quickly, reaching an observed maximum in August (Fig. 1).

Gonadal index — Gonad weights were converted to gonadal indexes (percent body weight) as used in other studies to compare fish of different lengths.

As reported by other workers (Vladykov 1956; Shiraishi and Fukuda 1966) for different areas, the ovarian index (Fig. 2) increased during the latter part of summer and into fall. The testicular index showed little increase until fall. However, although the July indexes were similar to that reported for immature gonads, the September indexes were below that reported for mature gonads. Nonetheless, in September the ovaries were laden with many large ova and were considered maturing; the testes were greatly enlarged as compared to their condition in July.

Discussion — This study showed that a gradual increase in photoperiod from winter to summer may not be important for stimulation of gametogenesis of brook trout in Pratt Lake. However, Baggerman (1957) found that a sharp change from short to long photoperiods stimulated gonad maturation in the spring-spawning *Gasterosteus* more than a gradual change from short to long. Thus, since brook trout are fall spawners, it is possible that the rapid change from near darkness to a long photoperiod stimulated gonad maturation of brook trout in Pratt Lake. Perhaps the rapid change of water temperature also influenced the stimulation of gametogenesis.

Decreasing fall day length and water temperature (similar to Henderson's experiment no. 13 with decreasing photoperiod and temperature) are probably important in the stimulation of final gonad maturation.

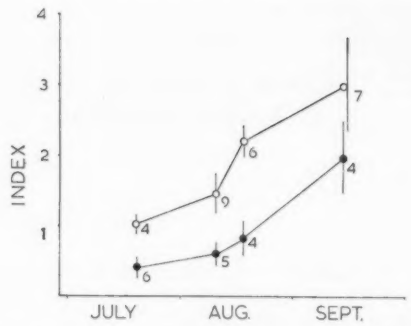

FIG. 2. Mean indexes (percent body weight) of gonads (± 1 standard error) of male (●) and female (○) brook trout in Pratt Lake in 1964. Numbers refer to sample sizes.

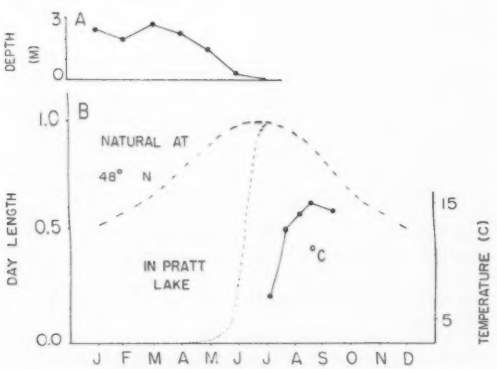

FIG. 1. A, estimated thickness of the winter cover on Pratt Lake in 1964. B, surface water temperatures, natural day-length cycle at 48° N (ratio of observed to maximum day length) and an estimate of the day-length cycle of brook trout in Pratt Lake in 1964.

Acknowledgments — I thank the following personnel of the College of Fisheries for their suggestions during the study: Dr A. Welander, Dr J. D. McPhail, Dr A. Seymour, Dr D. Greenfield, and Mr K. Mosher. The following read the manuscript and offered many valuable suggestions: Dr R. Drent, Dr M. Hughes, Dr T. G. Northcote, Dr C. Walters, Dr J. Bryan, and Mr W. Seim.

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Pectoral Fin Length in the Dogfish *Squalus acanthias* off British Columbia

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FORRESTER, C. R. 1972. Pectoral fin length in the dogfish *Squalus acanthias* off British Columbia. J. Fish. Res. Bd. Canada 29: 1211–1212.

In *Squalus acanthias* from three localities in British Columbia, mean lengths of the pectoral fin ranged from 85 to 93% of the nares–last gill slit length as compared to 76 and 106% for short-finned and long-finned forms of the species described in the literature.

FORRESTER, C. R. 1972. Pectoral fin length in the dogfish *Squalus acanthias* off British Columbia. J. Fish. Res. Bd. Canada 29: 1211–1212.

Nous avons étudié les caractéristiques de longueur de la nageoire pectorale de *squalus acanthias* des eaux de la Colombie-Britannique. La longueur moyenne de la nageoire varie de 85 à 93% de la longueur narine–dernière fente branchiale, comparativement aux valeurs de 76 et de 106% pour les formes à courte et à longue nageoire décrites dans la bibliographie de cette espèce.

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PECTORAL fin lengths of spiny dogfish (*Squalus acanthias*) in waters off Birtish Columbia were examined to determine the dogfish's taxonomic position as a long-finned (*S. acanthias suckleyi*) or short-finned (*S. acanthias acanthias*) type. This criterion together with fin-notching has been used to describe two subspecific groups (Lindberg and Legeza 1956, 1959).

Materials and methods — A total of 303 dogfish collected in 1971 from two localities in the Strait of Georgia and one in Queen Charlotte Sound were examined (Table 1). Length of the pectoral fin was measured from the last gill slit to the tip of the fin and compared with the distance from the last gill slit to the centre of the nares.

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Results and discussion — Relative lengths of the pectoral fin ranged from 74 to 100% of the nares–gill slit length with means of 85 to 93% among areas (Table 2). In no instance did the length of the fin exceed the nares–gill slit length. Regressoins of relative fin length against body length for males and females in the three localities showed a significant ($P = 0.05$) positive slope in all cases. Among areas there was no appreciable difference in slope but there was a significant difference in elevation of the regression lines (Table 2). However, the small numbers in some categories may have accounted for this difference. It was also noted that the distal margin of the pectoral fin was only slightly curved.

The short-finned type described by Lindberg and Legeza (1959) had pectoral fin lengths 60–90%

TABLE 1. Samples of *S. acanthias* collected in British Columbia waters in 1971 for pectoral fin measurements.

Area and gear	No. fish	% Male	Avg. length and range (*mm*) Male	Female
Strait of Georgia				
Tumbo Channel (otter trawl)	100	89	854(716–915)	888(726–1087)
Porlier Pass (gillnet)	101	78	843(720–915)	951(805–1135)
Queen Charlotte Sound (otter trawl)	102	50	795(480–898)	862(476–1117)

TABLE 2. Relative lengths of pectoral fin (as % of nares–gill slit length) and relations of fin to body length, by sex, in samples of *S. acanthias*.

Sample area	Sex	Relative length Mean	Range	Fin length–body length relation[a] Slope	Intercept
Tumbo Channel	Male	89.8	82.4–100.0	0.008	82.85
48°45′N, 123°15′W	Female	92.2	85.2–97.6	0.008	84.81
Porlier Pass	Male	90.2	81.8–100.0	0.022	71.57
49°N, 123°35′W	Female	92.5	86.5–100.0	0.034	60.38
Queen Charlotte Sound	Male	85.9	73.9–97.8	0.025	66.05
	Female	85.2	74.8–95.7	0.017	69.94

[a]Regression coefficients ($Y = a + bX$) for relative pectoral fin length (Y as %) on body length (X, cm).

(average 76%) of the nares–gill slit length and the long-finned type 101–110.5% (average 106%). Based on this criterion, the dogfish off British Columbia are intermediate to the two types. These authors reported that the short-finned type was found in the North Atlantic and in the Pacific in the southern part of the Sea of Okhotsk, Sea of Japan, and Yellow Sea; the long-finned type off Oregon, Washington, and California. They also reported that the short-finned type had an unnotched or slightly notched edge to the pectoral as compared to the clearly notched fin of the long-finned type.

At present, the American Fisheries Society (1970) lists the spiny dogfish as *S. acanthias* and makes no distinction between Pacific and Atlantic forms. The California dogfish, classified as *S. acanthias suckleyi* by Lindberg and Legeza (1959), has been noted as *S. acanthias* without further subspecific rank by Roedel (1953).

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Predation on Harbour Porpoise, *Phocoena phocoena*, by a White Shark, *Carcharodon carcharias*

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ARNOLD, P. W. 1972. Predation on harbour porpoise, *Phocoena phocoena*, by a white shark, *Carcharodon carcharias*. J. Fish. Res. Bd. Canada 29: 1213–1214.

A specimen of the white shark, *Carcharodon carcharias*, brought into Leonardville, Deer Island, N.B., on August 13, 1971, contained three harbour porpoises, *Phocoena phocoena*, in variable states of digestion. This record on predation is discussed in conjunction with literature reports of a similar nature and one other previously unpublished observation.

ARNOLD, P. W. 1972. Predation on harbour porpoise, *Phocoena phocoena*, by a white shark, *Carcharodon carcharias*. J. Fish. Res. Bd. Canada 29: 1213–1214.

Un requin blanc, *Carcharodon carcharias*, débarqué à Léonardville, Deer Island, Nouveau-Brunswick, le 13 août 1971, contenait trois marsouins communs, *Phocoena phocoena*, à divers stades de digestion. L'auteur examine ce cas de prédation en relation avec d'autres déjà signalés dans la bibliographie et avec un signalement encore inédit.

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ON August 13, 1971, a white shark, *Carcharodon carcharias*, was caught between Bliss and Whitehorse islands, at about 45°00′N, 66°52′W, and brought into Leonardville, Deer Island, southern New Brunswick for display. The specimen, examined by the author, was an estimated 5.2 m (17 ft) in total length, and weighed about 907 kg (2000 lb). Identification was based on size, deep body proportions, nearly homocercal tail, and the large, broadly triangular teeth with serrated margins. Two photographs were taken and are in the possession of the author. The specimen was later taken to the Biological Station of the Fisheries Research Board at St. Andrews, N.B., where identification was confirmed.

In the stomach of the specimen were three harbour porpoises (*Phocoena phocoena*) in variable states of digestion. The smallest animal (1.2 m, estimated) was almost entirely digested except for skeleton and tail stock. The largest animal (1.5 m, estimated) was fresh, with only slight digestion of integument; it was severed into three sections consisting of head (anterior to pectoral flippers), tail stock, and mid-portion containing most of the viscera. The left side was scored with parallel marks, apparently tooth scars. In all three animals the tail stock had been severed 0.2–0.5 m in front of the flukes, indicating that the attack regularly comes from behind.

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A 10-ft (about 3.1 m) white shark caught near Wallace, N.S., on July 30, 1962 had a "small porpoise" in its stomach (Templeman 1963). Day and Fisher (1954) reported an incident from the mouth of Passamaquoddy Bay, N.B., in which a "full grown" harbour porpoise, about to be shot, was bitten in half by a shark estimated to be over 14 ft (about 4.3 m) in length; the posterior half of the porpoise was swallowed. In the same area during September 1969, Messrs C. Hawes and R. Thurber, collecting for Dr D. E. Gaskin, also lost a porpoise to a large shark at least 4 m in length. This porpoise had been shot and wounded. Although specimens could not be taken in the above cases, the reported size and behavior excludes all species of shark known from inshore waters of the Canadian Atlantic except *C. carcharias*. Dr Gaskin also told the author that a 1.2 m female porpoise captured near Deer Island in August 1971 had a well-healed, deep, crescentic scar about 30 cm in length on the ventrolateral posterior portion of the left flank, very suggestive of a shark bite.

Bigelow and Schroeder (1948) showed that there were more reliable records of white shark from the Gulf of Maine than from any other comparable sector of the North American eastern seaboard, while a compilation of records from the Canadian Atlantic (Templeman 1963) showed that it is a regular visitor to these waters during the summer months, although not commonly captured. On the basis of these records and reports, the white shark

must be considered a potentially significant predator of harbour porpoise in the Canadian Atlantic.

Acknowledgments — I thank Dr D. E. Gaskin of the Department of Zoology, University of Guelph, and Dr A. W. Mansfield, Director of the Arctic Biological Station of the Fisheries Research Board at Ste. Anne de Bellevue, Que., for reading and commenting on this manuscript.

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Correction for Lag in Continuous-Flow Respirometry

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EVANS, D. O. 1972. Correction for lag in continuous-flow respirometry. J. Fish. Res. Bd. Canada 29: 1214–1216.

The lag and damping effect caused by fluctuations in the oxygen content of the respiration chamber in continuous-flow respirometry can be eliminated by addition of the simple correction factor (chamber volume × initial – final outflow oxygen concentration) to the ordinarily used formula (flow × mean difference between inflow and outflow oxygen concentration). Metabolism data for six rainbow trout, *Salmo gairdneri*, are presented.

EVANS, D. O. 1972. Correction for lag in continuous-flow respirometry. J. Fish. Res. Bd. Canada 29: 1214–1216.

Il est possible d'éliminer l'effet de décalage et d'amortissement que causent les fluctuations de teneur en O_2 dans la chambre respiratoire d'un appareil à flot continu. Il suffit d'ajouter un simple facteur de correction (volume de la chambre × teneur initiale–teneur finale en O_2 de la sortie d'eau) à la formule employée habituellement (débit × différence moyenne de teneur en O_2 de l'entrée et de la sortie d'eau). Nous donnons la valeur du métabolisme de six truites arc-en-ciel, *Salmo gairdneri*.

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CORRECTION for the level of random activity during metabolism studies is of considerable importance when, for example, animals are being compared under different temperature treatments, since random activity is differentially influenced at different temperatures, depending on past thermal history (Sullivan 1954; Peterson and Anderson 1969). Metabolism and activity can be correlated by measuring the oxygen removed from a closed chamber concurrent with measurement of activity (Beamish and Mookherjii 1964; Kutty 1968). However, there are many advantages to having the water flow through the chamber at some desirable constant rate (for review see Fry 1971). Consumption of oxygen over a given interval as calculated by the ordinarily used formula for continuous flow (Kausch 1968) cannot be applied as an exact measurement of oxygen consumed during the period if there are fluctuations in the metabolic rate such as occur during changes in the level of random activity. A lag will result in the oxygen consumption calculated because of the damping effect of the oxygen reservoir presented by the chamber itself. A suitable formula to remove the damping effect is presented by Fry (1971). The present data, which have been taken from a more complete study of rainbow trout metabolism (Evans MS 1971), illustrate the improvement brought about by the corrected formula.

Materials and methods — The respirometer was a closed, 4.48-liter, annular chamber. The single inlet was located in the bottom of the chamber and three outlets were spaced at 120° intervals around the top.

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The respirometer was housed in a circular bath from which it received an air-saturated, temperature-controlled water supply at constant head. The flow was adjusted so that oxygen concentration in the outflow was 1–3 mg/liter less than in the inflow and was about 11 liters/hr. The outflow passed over a polarographic electrode which was housed in a plexiglass jacket equipped with a magnetic stirring bar. The whole probe was mounted in a small water bath which received the overflow from the respirometer water bath.

Each fish was starved for 24 hr, placed in the respirometer, and allowed 36 hr to recover from handling. Fish were held at least 3 weeks at their acclimation temperature ±0.5 C before being tested.

Random swimming was monitored using a system of three lights and photocells spaced equidistantly around the respirometer (Kutty 1968). The number of times the light paths were broken was recorded for each 1-hr period. Oxygen consumption was determined by measuring the difference between inflow and outflow oxygen concentrations every 15 min. Oxygen consumed was calculated for each 1-hr period by two formulae: 1, the ordinarily used form $F(\overline{Y_1} - \overline{Y_2})$, where F = flow in liters and $(\overline{Y_1} - \overline{Y_2})$ is mean difference in oxygen concentration between inflow and outflow water in milligrams per liter; 2, incorporates the first but adds a correction factor, $V(Y_2t_0 - Y_2t_1)$, (Fry 1971), where V = volume of the respirometer – volume of the fish, Y_2t_0 and Y_2t_1 = oxygen concentration of outflow water at the beginning and end of the interval, respectively. Values derived from the above two calculations will henceforth be referred to, respectively, as uncorrected and corrected.

Results — The results are summarized in Table 1 and illustrated for one fish in Fig. 1 and 2. In Fig. 1, excursions of the corrected values are seen to be greater than those of the uncorrected ones. Corrected values also show an immediate response to sudden large changes in activity, while uncor-

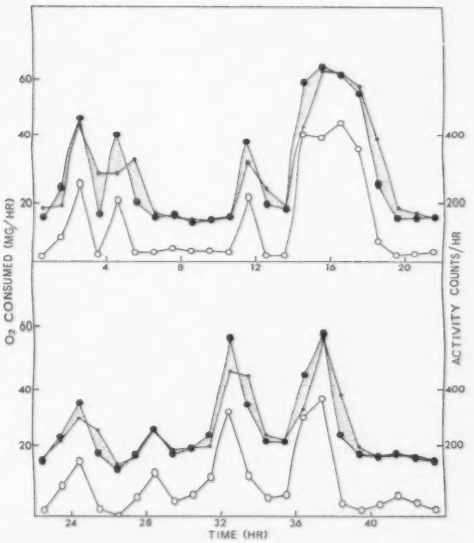

FIG. 1. Time course of activity (open circles) and oxygen consumption (uncorrected values, small closed dots; corrected values, large closed dots) of a single rainbow trout. Stippled area shows difference between corrected and uncorrected values.

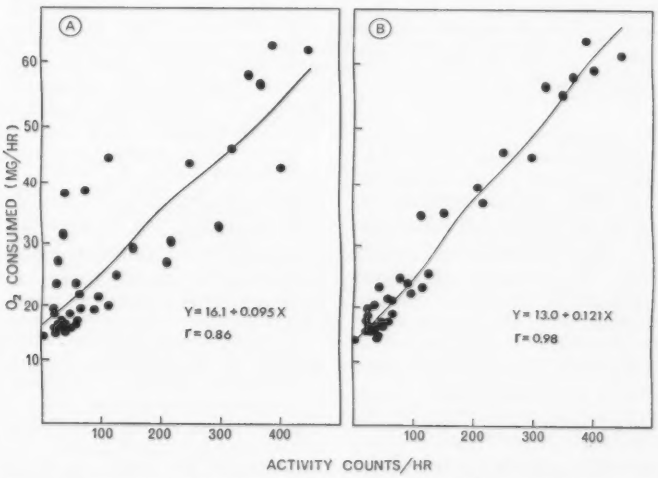

FIG. 2. Correlation of uncorrected (A) and corrected (B) oxygen consumption values with random locomotory activity.

TABLE 1. Summary of routine activity and oxygen consumption data for six rainbow trout.

Duration (*hr*)	Temperature (*C*)		Fish weight (*g*)	Regression equations		Correlation coefficients	
	Acclimation	Test		Uncorrected	Corrected	Uncorrected	Corrected
44	10	10	196	Y=16.1+0.095X	Y=13.0+0.121X	0.86	0.98
18	10	10	166	Y=8.8+0.141X	Y=6.0+0.178X	0.70	0.83
10	20	20	161	Y=22.6+0.012X	Y=17.3+0.042X	0.24	0.75
12	20	20	187	Y=30.0+0.051X	Y=27.3+0.058X	0.67	0.71
5	10	15	188	Y=31.1+0.081X	Y=28.4+0.094X	0.80	0.88
6	10	15	203	Y=42.7+0.061X	Y=33.6+0.079X	0.79	0.84

rected ones show a lag. Figure 2 shows that the deviations from regression are reduced and the slope is increased resulting in a lower estimate of standard metabolism when the correction is applied. The extrapolated oxygen consumption at zero activity with 95% confidence limits, using the uncorrected formula, is 16.1 ± 14.4 mg/hr compared to the corrected value of 13.0 ± 5.9 mg/hr. Similarly, at high activity, for example a count of 400/hr, the values are 54.1 ± 15.2 and 61.4 ± 6.2 mg/hr, respectively.

Table 1 shows that in all cases the regression coefficients are increased and the correlations improved by use of the corrected formula. In some cases the range of activity for a particular fish did not justify extrapolation to zero activity as an estimate of standard metabolism. In the original analysis (Evans MS 1971), standard values were estimated by pooling fish for similar treatments. In this case, however, individual fish are presented only to illustrate the correction. The Y intercept values shown in Table 1 are not necessarily good estimates of standard metabolism.

Discussion — The calculation of oxygen consumption by the uncorrected formula produces an apparent time lag and often only a weak correlation between activity and oxygen consumption. In addition, the extreme high and low values of oxygen consumption are dampened. Time lag, apparently of this type, has recently been interpreted as oxygen debt by Kausch (1968) in his studies of the respiration of carp. Unfortunately, the correction has been overlooked by most workers. This is ironical since the often quoted work of Spoor (1946), who pioneered the method of measuring activity simultaneously with oxygen consumption, states briefly but clearly that "calculations took into account the change in the amount of oxygen in the constant volume of water in the chamber" when using continuous-flow respirometry.

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Influence of Salinity, Temperature, and Exercise on Plasma Osmolality and Ionic Concentration in Atlantic Salmon (*Salmo salar*)

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Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

BYRNE, J. M., F. W. H. BEAMISH, AND R. L. SAUNDERS. 1972. Influence of salinity, temperature, and exercise on plasma osmolality and ionic concentration in Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 29: 1217–1220.

In unexercised Atlantic salmon (*Salmo salar*), plasma osmolality and Na^+ and Ca^{++} concentrations varied little at temperature-salinity combinations of 3, 5, 10, and 14 C and 0, 15, and 30‰. Plasma K^+ tended to increase with increase in temperature. Cl^- values were similar at 5 and 14 C. At 1 C and 0‰, lower plasma osmolalities indicated reduced ability to osmoregulate. At 1 C and 30‰, elevated osmolalities and ionic values and mortalities indicated severe osmotic stress.

After exercise for 2 hr at 3–4 body lengths/sec at 5 and 14 C, salmon in salinities of 0 and 15‰ had osmolalities and ionic values the same as or little changed from unexercised values. There were marked increases in the values following exercise in 30‰.

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Chez des saumons atlantiques (*Salmo salar*) maintenus au repos, l'osmolarité plasmatique et la teneur en Na^+ et Ca^{++} varient peu à des combinaisons de températures et de salinités de 3, 5, 10, et 14 C et 0, 15, et 30‰. Le K^+ plasmatique a tendance à augmenter avec la température.

La teneur en Cl^- demeure la même à 5 et à 14 C. A 1 C et 0‰, le pouvoir osmorégulateur semble diminuer, comme le suggère une osmolarité plus basse. A 1 C et 30‰, l'augmentation de l'osmolarité, des valeurs d'ionisation et des mortalités démontre un stress osmotique aigu.

Après exercices de 2 h à une vitesse de 3 à 4 longueurs de corps par seconde à 5 et 14 C, l'osmolarité et les valeurs d'ionisation des saumons exposés à des salinités de 0 et 15‰: diffèrent peu ou pas du tout de celles des saumons maintenus au repos. L'activité à des salinités de 30‰ produit un accroissement marqué de ces paramètres.

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PLASMA osmolality and ionic composition were determined for unexercised and exercised postsmolt Atlantic salmon (*Salmo salar*) in relation to temperature and salinity. Influence of salinity on plasma composition in this species has been investigated by Parry (1966), Houston and Threadgold (1963), and Potts et al. (1970). However, information is not available on plasma composition of Atlantic salmon either at low temperature or under stress of severe exercise. At low temperatures, ionic imbalance has been demonstrated for Atlantic cod, *Gadus morhua* (Woodhead and Woodhead 1965), and for crucian carp, *Cyprinus carassius* (Wikgren 1953). Osmotic concentration of rainbow trout, *Salmo gairdneri*, increased following exercise over a range of salinities (Rao 1969).

Materials and methods — Hatchery-reared salmon (15–20 cm) were brought in the spring to the Biological Station of the Fisheries Research Board of Canada, St. Andrews, N.B., and were held in running fresh or sea water in fiberglass tanks for periods of 6–18 months

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before sampling. At sampling, all of the salmon were post-smolts. They were fed daily a mixture of ground beef liver, Atlantic cod fillets, and Purina trout chow (42.5, 42.5, and 15% by weight, respectively).

To obtain desired salinity a daily increment of 7–10‰ was made. Once the desired salinity was reached, temperature was changed by 1 C/day until the experimental temperature was reached. Fish were maintained at the desired salinity for at least 2 months and at the desired temperature for at least 2 weeks before being used in experiments.

Most blood samples were taken from unanesthetized fish. Tertiary amyl alcohol was used as an anesthetic in some experiments. This resulted in osmolalities being about 11% higher than when no anesthetic was used. Concentrations of individual ions were the same in anesthetized and unanesthetized fish. It is thought that TAA entered the blood and acted as an antifreeze, thus lowering the freezing point and giving anomalous osmolality. Suitable corrections were made for osmolality when TAA was used.

Blood was taken by cardiac puncture, each fish being sampled once. Syringes were chilled and lubricated with paraffin oil, but no anticoagulant was used. Clotting rarely occurred. The blood was centrifuged at about 1500 rpm for 3 min and plasma osmolality and chloride concentrations determined immediately. Chloride concentrations for unexercised fish were determined only at 5 C in 15 and 30‰, and at 14 C in 0, 15, and 30‰. A portion of the plasma was immediately freeze-dried and stored at room temperature for future determination of Na^+, K^+, and Ca^{++} concentrations at the University of Guelph.

At Guelph, the freeze-dried samples were dissolved in double-distilled water to which 0.1 N HCl and 0.02% wetting agent were added. Na^+ and K^+ concentrations were determined by flame emission spectroscopy and Ca^{++} by the atomic absorption method. Because flame spectra of ions have several peak wavelengths, the possibility was checked of single ions and combinations of ions influencing readings for another ion. One interference was discovered. Na^+ in samples produced a higher reading for K^+ than if K^+ was determined alone or in the presence of Ca^{++}. Because of this effect, Na^+ concentration of standards was made similar to that of samples.

Possible interferences with Ca^{++} determinations by phosphate and protein were checked by comparing Ca^{++} results for duplicate samples, one of which was diluted with releasing agent (0.1% $Sr\,Cl_2$), 0.1 N HCl, and 0.02% wetting agent. Releasing agent was not added to the other sample. Calcium values were the same with and without releasing agent, so it was not used for subsequent determinations.

To determine if freeze-drying affected results for cation concentrations, 12 paired samples of fresh and freeze-dried plasma of carp (*Cyprinus carpio*) were compared. Na^+, K^+, and Ca^{++} values were similar at $P = .10$ (paired *t*-test).

Precision in terms of range was ±0.7% for the osmometer, ±1.5% for the chloridometer, and ±2.5% for the spectrophotometer based on triplicate analyses of standard solutions.

Since the salmon were sampled over a long period, there was a wide range in fish size. Accordingly, the effect of fish size on plasma osmolality and ionic concentrations was examined. Only at 14 C, where the widest size range was available, was it possible to demonstrate a size effect. There was a linear increase in Ca^{++} values with increasing weight; Na^+, Cl^-, and K^+ values and osmolality were independent of weight. Regression equations describing changes in Ca^{++} with weight were used to correct Ca^{++} values for a weight of 200 g, the approximate mean weight of fish used. Osmolality and ionic values did not differ for males and females at given temperatures and salinities.

To study changes in plasma osmolality and ionic concentration in relation to exercise, a Blazka type of activity chamber (Beamish 1966) was employed. Individual fish were subjected to low water velocity until they oriented into the current. Water velocity was then increased gradually to between 70 and 100 cm/sec (about 3–4 body lengths/sec). Salmon were forced to swim for 2 hr, after which most were exhausted.

Means and standard errors were calculated for length, weight, and plasma concentrations. If means ±2 SE (95.4% confidence limits; Alder and Roessler 1964) overlapped, it was concluded that no significant differences existed.

Results and discussion — The most striking feature of the plasma osmolality and ionic measurements from salmon was their relative constancy over wide salinity and temperature ranges. In unexercised salmon, osmolality and Na^+ values were little affected by salinity over the temperature range 3–14 C (Fig. 1). At 1 C, osmolality at 0‰ was significantly lower than that at higher temperatures, an indication of possible osmotic imbalance. However, ionic values at 1 C and 15‰ were similar to those at higher temperatures. At 1 C and 30‰ both osmolality and Na^+ values were significantly higher than those observed for unexercised salmon at any other temperature or salinity used. That 7 of 30 fish held at 1 C and 30‰ died during the experiment indicates severe stress because of osmotic imbalance. Mortality of Atlantic cod at low temperature has been attributed to osmotic imbalance by Woodhead and Woodhead (1965). Presumed osmotic imbalance in salmon at 1 C at 0 and 30‰ appears to have been influenced by an interaction between salinity and temperature rather than by temperature alone. At 1 C and 15‰, the salinity closest to an isosmotic medium of 10‰, osmotic and Na^{++} values were similar to those at 3–14 C and the three salinities. Brett (1952) and Doudoroff (1945) have reported an increase in survival time at low temperatures when fish were held at or near the isosmotic salinity.

Plasma K^+ values increased with increase in temperature at 0 and 15‰ (Fig. 1) and were in agreement with those of Houston et al. (1968) who found

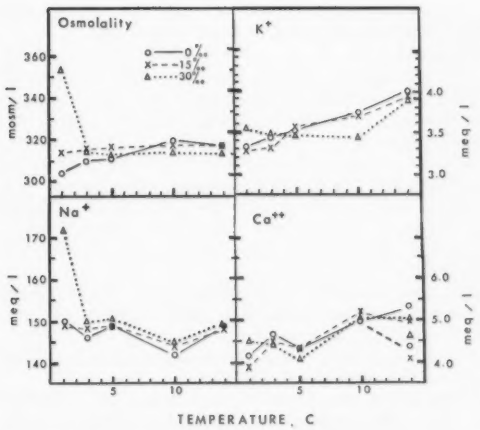

FIG. 1. Variation in plasma osmolality and ionic concentrations with temperature for unexercised salmon held for at least 2 months at three salinities and for at least 2 weeks at four temperatures. Numbers of salmon: 1, 3, and 5 C, 10–17 for osmolality, 5–14 for ionic concentrations; 10 and 14 C, three to seven for both osmolality and ionic concentrations except for two fish at 14 C and 15‰. For Ca^{++} values at 14 C, dashed line indicates values corrected for weight effect.

a trend of increasing K^+ and Ca^{++} values in rainbow trout with increase in temperature. At 30‰, K^+ values were about the same between 1 and 10 C and lower than at 14 C. Except at 10 C and 30‰, K^+ values were similar among the three salinities at each temperature. Increased plasma K^+ in Atlantic salmon at higher temperatures is consistent with the report by Hickman et al. (1964), who noted that cell K^+ in frog and mammalian muscle cells apparently is subjected to increased bonding to protein at low temperature. Moreover, Davson (1964) and others have demonstrated that the erythrocyte membrane is less permeable to K^+ at low than at high temperatures.

There was no consistent trend in Ca^{++} values with respect to temperature (Fig. 1). At a given temperature, Ca^{++} values were not significantly different among salinities.

For unexercised fish, plasma Cl^- did not vary significantly with temperature or salinity at the temperatures and salinities used (Fig. 2).

After exercise in salinities of 0 and 15‰, generally there was little change in osmolality and ionic concentrations from those of unexercised salmon at 5 and 14 C (Fig. 2). Plasma Cl^- was lower, however, for exercised fish at 0‰. At 30‰ and at both 5 and 14 C, for exercised fish osmolality and ionic values were higher, excepting Ca^{++} at 14 C. Intake of sea water is probably responsible for the large increases in Na^+ and Cl^- concentrations (and hence osmolality) for salmon exercised in 30‰. Stevens (1968) reported that rainbow trout had gained weight after exercise in fresh water and concluded that fish took in water.

At 15‰, the test salinity closest to the isosmotic medium of 10‰, plasma osmolality and ionic concentrations for exercised salmon were similar to those of unexercised fish. Rao (1968) and Farmer and Beamish (1969) have demonstrated with rainbow trout and *Tilapia nilotica*, respectively, that, for a given swimming speed, oxygen consumption was lowest at or near the isosmotic salinity. In salinities above or below the isosmotic level, oxygen consumption increased. Rao found that oxygen consumption associated with a given swimming speed was independent of salinity, and he ascribed the increased O_2 consumption to cost of osmoregula-

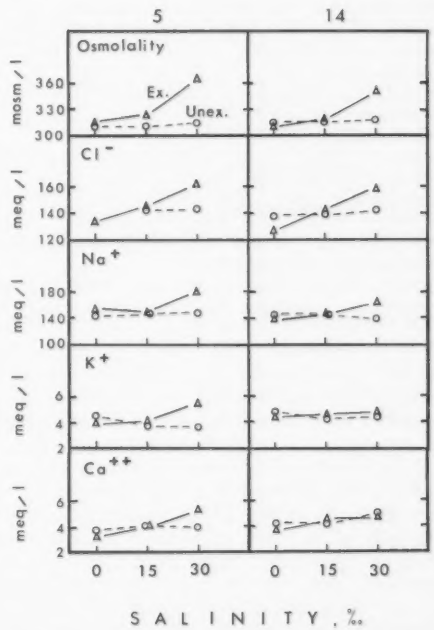

FIG. 2. Plasma osmolalities and ionic concentrations of unexercised and exercised salmon held for at least 2 months at 0, 15, and 30‰ and for at least 2 weeks at 5 and 14 C. Numbers of exercised salmon: 5 C, four to nine except for three fish at 30‰ for ionic concentrations; 14 C, 7–10 fish; of unexercised salmon: 5 C, 6–15 except for two fish at 15 and 30‰ for Cl^-; 14 C, four to six fish at 0 and 15‰ and two at 30 .

tion. It appears that ability of salmon to osmoregulate in different salinities parallels cost of osmoregulation. Thus, exercised salmon at 15‰ maintained plasma concentrations most similar to those for unexercised fish, followed by progressively greater deviation for exercised fish at 0 and 30‰. Rao (1968) and Farmer and Beamish (1969) have shown that cost of osmoregulation for swimming fish is less in 0‰ than in 30‰.

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An Analysis of Temperature Effects on the Inshore Lobster Fishery[1]

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In the past, water temperature has been utilized in combination with some measure of fishing effort in the development of economic estimator or predictor equations for the yield of the lobster *Homarus americanus*. The hypothesis that the inshore lobster fishery in the United States has been overfished since the end of World War II to the point where increases in fishing effort since that time have had only minor effects on the yields was examined. It was shown that suitable yield prediction equations could be developed using only lagged and present temperatures as the independent variables. Comparisons were made of equations developed for the Maine fishery and sections of the Canadian fishery. Further analyses were done comparing equations developed using winter vs. summer temperatures and surface vs. bottom temperatures.

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La température de l'eau, couplée à des mesures d'effort de pêche, a souvent été utilisée dans des équations servant à estimer ou à prédire le rendement de la pêche au homard, *Homarus americanus*. Nous examinons l'hypothèse suivante: depuis la fin de la Deuxième Guerre mondiale, la pêche au homard sur le littoral des Etats-Unis a été trop intensive, au point qu'une augmentation de l'effort de pêche n'a eu depuis ce temps que des effets minimes sur le rendement. Nous démontrons la possibilité de développer des équations pour prédire le rendement de la pêche en utilisant, comme seules variables indépendantes, les températures antérieures et les températures présentes. Nous comparons les équations applicables aux pêches du Maine avec celles de certaines sections des pêches canadiennes. Poussant l'analyse plus loin, nous comparons des équations basées sur les températures d'hiver par rapport aux températures d'été et sur les températures de surface par rapport aux températures de fond.

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MUCH has been written concerning the effects of effort and temperature on the variation in catch of the American lobster (*Homarus americanus*). McLeese and Wilder (1958), Dow (1966, 1969), and, more recently, Cooper and Uzmann (1971) have written on various aspects of temperature and its effect on the lobster. Dow (1966) has argued that temperature and fishing effort have been largely responsible for fluctuations of the lobster catch in Maine. He implied further that the close correlation between the fluctuations of the Maine lobster fishery and other lobster fisheries along the Atlantic seaboard indicate these same factors prevail elsewhere. A Bureau of Commercial Fisheries study group (unpublished data) concluded that the decline in the U.S. coastal catch was partly, if not largely, associated with a long term trend of declining water temperature. Coupling these arguments with the results of a recently completed computer simulation modeling study of a hypothetical lobster fishery (unpublished data) has led to a closer investigation of empirical yield prediction.

Fishing effort measured by the number of traps for the Maine lobster has increased 199.2% from 1944 to 1964 (Dow 1969), and in successive years has continued to increase. Dow noted that after 1956 effort and catch were inversely related in the Maine fishery as well as in the New York–Newfoundland fisheries. Under the hypothesis that the Maine fishery has been oversaturated with fishing effort since the end of World War II, effort as a variable contributing to the fluctuation of yield may be held constant. Hence, temperature alone may be used in the development of equations estimating the yield of the fishery.

Methods — From the simulation model previously mentioned, basic relations have been established and

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some of these will be briefly described. Any stimulation of the lobster fishery, whether induced by man or the environment, will affect the output of the fishery in three phases: (a) an immediate rise or drop in catch, followed by a gradual rise or decline depending on the magnitude of the initial stimulation; (b) a sharp rise or decline in catch 6–8 yr after the initial stimulation, followed by a gradual rise or decline; (c) a sharp rise or decline in catch 13–15 yr after the initial stimulation. This peak or dip will not be as pronounced as the 6–8-yr effect.

Using these basic relations, stepwise multiple regression equations were developed on the University of Rhode Island IBM 360/Mod 50 computer using the variables described below. In the development of the regressions, the independent variables were added to the regression in a descending order of importance, based on the reduction of the total sums of squares.

The variables for each regression were entered in the following order: 1, Y_o = present year's yield (dependent variable); 2, Y_{-n} = previous annual yields (independent variable) $n = 1,\ldots,8$; 3, T_o = present mean annual temperature (independent variable); 4, T_{-n} = previous mean annual temperature (independent variable) $n = 1,\ldots,8$.

If yield Y_0 is some function of a combination of effort and temperature, then previous yields should have a weighting effect on present yields. Hence, previous yields were used in the regressions to test the validity of holding effort constant. Lagging combinations of previous yields and temperatures forward and backward in time from the 6–8-yr expected effect and backward from the present effectively tested the model relations (a) and (b), and the dominance of catch variation due to temperature over variation due to effort was demonstrated. Further analyses were carried out to determine if the high summer temperatures during which larval development takes place were more significant than the low winter temperatures following larval development in estimating yield.

Results and discussion — Figures 1 and 2 are the graphical representations of temperature and catch data used in the analyses. The analyses for Maine and Nova Scotia data are contained in Tables 1 and 2. In the development of each equation the deviations from the regression line were tested for normality about the regression line. These conditions were satisfied.

Equations 1 and 2 — A large proportion of the lobster catch in both the U.S. and Canada consists of newly recruited lobsters. Dow (1966) indicated that some 90% of the total catch for Maine was composed of new recruits and considered temperature as the controlling factor in the molting of the lobster. McLeese and Wilder (1958) emphasized the importance of temperature on the activity and catchability of the lobster. Hence, it would seem reasonable to assume that present temperature T_o would be an effective indicator of current yield. In the stepwise multiple regression performed on the Maine data, T_o was found to be significant but second in importance to the lagged temperature combination of T_{-6}, T_{-7}, and T_{-8}. Of the latter three, T_{-6} was the most significant contributor. From the analysis of winter and summer temperatures it was determined that winter temperatures were more significant for the development of yield estimation equations. For T_{-678} this may indicate high first year mortality during the colder winter months and for T_o less recruitment into the fishery. Working with these results it was found that log (T_{-678}) improved the regression and allowed the development of equation 2, using only the coldest months for T_{wo}. A comparison of equation 1 with equation 2 is indicative of the reliability of the winter temperatures but is more indicative of the weight carried by log (T_{-678}). Using equation 2, suitable estimates

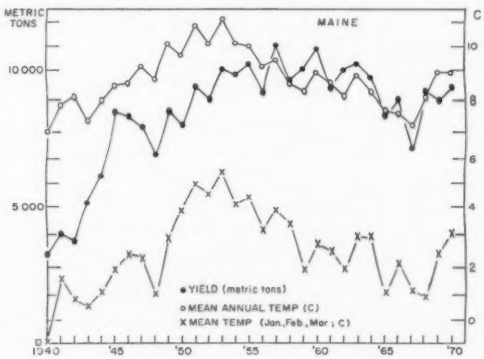

FIG. 1. Annual yield (modified after Dow 1969), mean annual surface temperature, mean surface temperature, C, (January, February, March) for Maine.

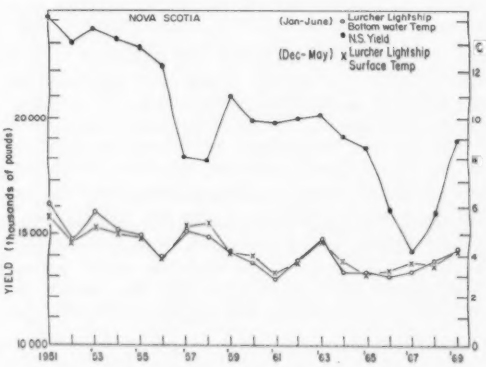

FIG. 2. Annual yield, (*thousands of pounds*) mean bottom temperature (January–June) Lurcher Lightship, mean surface temperature, *C*, (December–May) Lurcher Lightship, for Nova Scotia.

TABLE 1. Summary of Maine yield estimation equations obtained by stepwise multiple regression.

T = surface temperature *C* (Boothbay Harbor), 1939–70
Y = yield (*metric tons*), 1947–70

$$Y_o = -19599.203 + 17238.324 \log (T_{-678}) + 485.573\ T_o \quad (1)$$

Multiple correlation coefficient, r = 0.874 (adjusted r = 0.868)
F for A.N.O.V. (df = 2, 21), 33.947
SE of estimate, 493.777 (adjusted SE = 504.874)
Y_o = present year's yield (*metric tons*)
T_{-678} = (T_{-6} + T_{-7} + T_{-8}) sum of mean annual temperatures for 6, 7, and 8 yr previous to T_o
T_o = mean annual temperature of present year

$$Y_o = -12680.965 + 14740.687 \log (T_{-678}) + 345.823\ T_{wo} \quad (2)$$

Multiple correlation coefficient, r = 0.85 (adjusted r = 0.843)
F for A.N.O.V. (df = 2,21), 27.446
SE of estimate, 534.407 (adjusted SE = 546.417)
Y_o = present year's yield (*metric tons*)
T_{-678} = (T_{-6} + T_{-7} + T_{-8}) sum of mean annual temperatures for 6, 7, and 8 yr previous to T_o
T_{wo} = mean temperature for the months of January, February, and March (present year)

may be made of the present year's catch at the end of March for Maine.

Equation 3 — From an objective examination of this equation, developed from long term data, the following points merit some mention: the appearance of Y_{-1} as an independent variable for yield prediction may indicate a long term effort fluctuation or may be a reflection of T_{-1}; T_{-1} could be an indicator of this year's yield for the following reason. The Nova Scotia fishery is primarily a spring fishery ending on or about the last day of June and may be dependent on recruitment which took place the previous year; this equation is not in agreement with the equations developed for Maine. A short-term equation was also developed using these data and the same variables dominated. The St. Andrews surface water is well mixed with the bottom water (D. G. Wilder personal communication). This may make the surface temperature data more variable making the long term record noisy. Working under this assumption, equation 4 was developed.

Equation 4 — Using the surface water temperature of the Lurcher Lightship (Fig. 2), this equation was developed as a comparison for the equation developed using the St. Andrews temperature data. It does not agree with the St. Andrews equation but is in complete agreement with the Maine equations 1 and 2.

Equation 5, 6, and 7 — Using the bottom water temperature at the Lurcher Lightship (50 fathoms), equation 5 was developed. A noticeable increase in the goodness of fit may be observed in comparison with equations 1 and 4. To emphasize the importance of T_{-6}, equation 6 was developed. Using only the lagged temperature, estimates of yield can be made up to 1975 as shown in Table 3. The observed catch for 1970 and 1971 was not used in the development of the equation. The reliability of any equation developed for estimation or prediction is a test using data not included in the development of the equation.

Due to the increased goodness of fit when the bottom water temperature of the Lurcher Lightship was used, equation 7 was developed from the mean for the months January–April for T_{wo} allowing for a reasonable estimate of the Nova Scotia catch for the present year and an increase in the overall accuracy of equation 6 on a short-term basis. Unfortunately, the Lurcher Lightship has been removed and bottom water temperatures are no longer available from this area.

Conclusions — Overall agreement of the equations developed using post World War II catch data and the lack of dependence of yield on an effort function reinforces the hypothesis of a constant effort function. If, in future use of these estimators, the estimates of yield are consistently higher than the observed yields, this may indicate a negative effort function. That is, effort as a factor contributing to the yield of the fishery may be such that its only effect is detrimental to the fishery. On the other hand, if the estimates made are consistently lower than the observed yield, the effort function will become a positive function or contributor to the success of

TABLE 2. Summary of Nova Scotia yield estimation equations obtained by stepwise multiple regression.

T = surface temperature *C* (St. Andrews), 1921–70
Y = yield (*thousands of pounds*), 1929–70

$$Y_o = -2775.769 + 0.64246\ Y_{-1} + 1329.426\ T_{-1} \quad (3)$$

Multiple correlation coefficient, r = 0.825 (adjusted r = 0.821)
F for A.N.O.V. (df = 2, 39), 41.674
SE of estimate, 1802.281 (adjusted SE = 1824.67)
Y_o = present year's yield (*thousands of pounds*)
Y_{-1} = previous year's yield (*thousands of pounds*)
T_{-1} = mean annual temperature, previous year

T = surface temperature (Lurcher Lightship), 1951–69
Y = yield (*thousands of pounds*), 1959–69

$$Y_o = -22960.273 + 29850.929 \log (T_{-678}) + 2033.623\ T_o \quad (4)$$

Multiple correlation coefficient, r = 0.861 (adjusted r = 0.844)
F for A.N.O.V. (df = 2,8), 11.478
SE of estimate, 1229.671 (adjusted SE = 1296.185)
Y_o = present year's yield (*thousands of pounds*)
$T_{-678} = (T_{-6} + T_{-7} + T_{-8})$ sum of mean temperatures (December-May) for 6, 7, and 8 yr previous to T_o
T_o = mean temperature (December–May) present year

T = bottom temperature *C* (Lurcher Lightship), 1951–69
Y = yield (*thousands of pounds*), 1959–69

$$Y_o = -18316.285 + 27314.555 \log (T_{-678}) + 1626.852\ T_o \quad (5)$$

Multiple correlation coefficient, r = 0.943 (adjusted r = 0.937)
F for A.N.O.V. (df = 2,8), 32.373
SE of estimate, 802.156 (adjusted SE = 845.545)
Y_o = present year's yield (*thousands of pounds*)
$T_{-678} = (T_{-6} + T_{-7} + T_{-8})$ sum of mean temperatures (January–June) for 6, 7, and 8 yr previous to T_o
T_o = mean temperature (January–June) present year

$$Y_o = 4470.898 + 21834.949 \log (T_{-6}) \quad (6)$$

Multiple correlation coefficient, r = 0.873 (adjusted r = 0.873)
F for A.N.O.V. (df = 1,9), 28.844
SE of estimate, 1112.154 (adjusted SE = 1112.154)
Y_o = present year's yield (*thousands of pounds*)
T_{-6} = mean temperature (January–June) for sixth year previous to T_o

$$Y_o = -18024.262 + 27692.621 \log (T_{-678}) + 10661.926 \log T_{wo} \quad (7)$$

Multiple correlation coefficient, r = 0.906 (adjusted r = .895)
F for A.N.O.V. (df = 2,8), 18.393
SE of estimate, 1022.335 (adjusted SE = 1077.635)
Y_o = present year's yield (*thousands of pounds*)
$T_{-678} = (T_{-6} + T_{-7} + T_{-8})$ sum of mean temperatures (January–June) for 6, 7, and 8 yr previous to T_o
T_{wo} = mean temperature (January–April) present year

TABLE 3. Observed and estimated yield (*thousands of pounds*) for Nova Scotia using equation 6.

Year	Observed (N.S.)	Estimated (N.S.)	Residual
1970	15787	16019	+232
1971[a]	13619	15199	+1580
1972		14951	
1973		15935	
1974		17180	
1975		18412	

[a]As of November preliminary estimate

the fishery. Caution must be taken in using these as a guide since Dow (1969) has pointed out that effort and catch after 1956 are inversely related for the Maine fishery as well as the New York–Newfoundland fisheries. This being the case, a slightly negative effort function may have been unwittingly incorporated into the equations.

A comparison of the equations developed using surface water temperature to those developed using bottom water temperature (Lurcher Lightship) leaves little doubt as to the desirability of collecting bottom water temperatures for this particular type

of analysis. Although surface water temperatures can be used to develop yield estimation equations, there is enough variation in the surface record to decrease the accuracy of the estimations. For the bottom water records, the variation or noise is smoothed to the extent that the yield estimations become considerably more accurate.

Acknowledgments — Temperature data utilized in the analysis was provided by the National Marine Fisheries Service at Booth Bay Harbor, Maine. Catch data was taken from Dow (1969) and supplemented with data from J. Thomas, Maine Department of Sea and Shore Fisheries.

All data used in the analysis for the Canadian Provinces was obtained through the courtesy of Dr D. G. Wilder, Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B.

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Acute Toxicity of Spent Sulphite Liquor to Atlantic Salmon (*Salmo salar*)

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WILSON, R. C. H. 1972. Acute toxicity of spent sulphite liquor to Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 29: 1225–1228.

Ninety-six-hour static bioassays, with daily toxicant replenishment, were performed on Atlantic salmon (*Salmo salar*) parr to test for the acute toxicity of spent sulphite liquor (SSL). The LC50 of two batches of fresh SSL varied from 2000 to 2400 ppm, while the LC50 of SSL which had been previously oxidized for 5 days was 2750 ppm. Test fish responded differently to oxidized than to fresh SSL. Caution is urged in the application of 96-hr bioassay results on sulphite pulp mill wastes to field situations.

WILSON, R. C. H. 1972. Acute toxicity of spent sulphite liquor to Atlantic salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 29: 1225–1228.

Des essais biologiques statiques de 96 heures, avec recharge quotidienne du toxique, ont été effectués sur des tacons de saumon atlantique (*Salmo salar*) en vue de mesurer la toxicité aiguë de la liqueur usée de sulfite (SSL). La LC50 de deux lots de SSL fraîche a varié de 2000 à 2400 ppm, alors que celle de la SSL préalablement oxydée durant 5 jours a été de 2750 ppm. Les poissons d'essai ont réagi de façon différente à la SSL oxydée et fraîche. On devra donc appliquer avec circonspection aux conditions sur le terrain les résultats d'essais biologiques de 96 heures avec les liqueurs résiduaires de papeterie.

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THE sulphite pulping process is of major importance in the production of paper in eastern North America. Although there are at least nine mills using this process in the Maritime provinces alone, little information is available on the effects of sulphite wastes on the environment. The work reported here is an attempt to determine the acute toxicity of spent sulphite liquor (SSL) to Atlantic salmon (*Salmo salar*) in a standard 96-hour bioassay, and forms part of a program to determine the toxicity of industrial wastes in the Exploits River, Nfld.

Since pulp mills are usually situated beside a body of water, their effluent is often discharged into the water. The discharge of SSL is known to affect the natural *p*H, BOD, COD, and transparency of a receiving water (Bagge 1969). These effects reflect the essential characteristics of the liquor; it contains a substantial amount of organic matter, which causes turbidity in its native state and oxygen depletion during its degradation, and it contains a quantity of acidic hydrogen ions.

In estuarine water, the toxicity of SSL appears to vary with the type and developmental stage of the organism tested. Woelke (1968) reported that the development of fertilized eggs of the Pacific oyster was affected at a level as low as 3 ppm, while herring eggs have hatched successfully at concentrations up to 27.2 ppm (Holland et al. 1960). In tests on three species of Pacific salmon, the action of SSL was found to depend on the age of the fish, with younger fish being more resistant. The 30-day LC50 varied also with species but ranged from 1550 ppm for 102-day-old pink salmon to 530 ppm for 198-day-old pink salmon (Williams et al. 1953). In a test in fresh water, Grande (1964) found that about 600 ppm was completely lethal to brown trout in 9 days.

Methods — Four static bioassays were performed on Atlantic salmon parr, involving a total of 45 tanks, each of which contained 10 fish. The first three experiments tested fresh SSL, while the fourth tested SSL which had been oxidized by bubbling air at the rate of 1 liter/min for 5 days through a 5-liter sample held at 16–18 C. The toxicant was obtained from the Price (Nfld.) Ltd. mill located on the Exploits River at Grand Falls by drawing off a 12-liter sample from a digester immediately following each of two cooks. Both samples were then split into 1-liter aliquots and frozen in plastic bottles until required. Salmon parr were caught by electrofishing wild populations in streams of central Newfoundland, outside the influence of industrial effluents, and acclimated for a week in the test water. Following this, they were screened for size uniformity and obvious physical defects. Only one control mortality resulted from this technique in a series of 10 tests involving more than 150 control fish, and a fairly even size distribution around a mean length of 8.5 cm was obtained.

Details of each experiment were:

	Test			
	1	2	3	4
SSL batch no.	1	1	2	2
condition	fresh	fresh	fresh	oxidized
Concn range (*ppm*)	22–1015	29–5150	17–4900	17–4786
Init. hardness (*mg/liter*)	9	8	9	9
Initial *p*H	6.5	6.6	6.7	6.7
Initial alk. (*mg/liter*)	4	6	8	8
Test temp (*C*)	16.6 (±0.5)	18.0 (±0.2)	16.4 (±0.4)	16.4 (±0.4)
Fish length (*cm*)	7.5 (±1.6)	8.0 (±2.5)	8.4 (±1.3)	9.7 (±1.9)

There was a small variation in test temperatures between about 16–18 C, which resulted from a desire to conform as closely as possible to the seasonal ambient. Illumination in the bioassay room was provided solely by fluorescent lamps, which were turned on and off to give a day length of about 13 hr. SSL concentrations were obtained from the analysis of water samples taken from the bioassay tanks at the beginning of each 24-hr period, using the modified Pearl-Benson Index (Barnes et al 1963). This index (P.B.I.) measures the intensity of the color developed by a nitroso-lignin complex, and relates it to the color developed by standard solutions of SSL whose volume has been adjusted to a 10% solids basis.

Toxicant solutions were mixed into aerated, polyethylene-lined tanks by adding the SSL to 50 liters of water taken from Exploits River above the pulp mill. The solutions and the tank linings were replaced daily to minimize toxicant loss due to oxidation and to reduce tank effects. Ten salmon parr were randomly assigned to each test tank, and observations on their behavior were made at the end of each hour for the first 10 hr of exposure, at 15 and 20 hr, and at the end of each 10-hr period thereafter.

To test for the possible importance of a decline in the SSL concentrations by oxidation and adsorption between water changes, samples of the test water were analyzed before and after each change during tests 3 and 4. Replicate concentrations showed an average fluctuation of 4.2% about the mean value, which was more than the apparent reduction observed over a 24-hr period. It is unlikely that such a small fluctuation materially affected the results.

Results and discussion — Figure 1 shows that the higher SSL concentrations were fairly acid. In addition, it was difficult to maintain the dissolved oxygen in these tanks at satisfactory levels, in spite of vigorous aeration. Both dissolved oxygen and *p*H fell to minima of 3.5 mg/liter and *p*H 4.82, respectively,

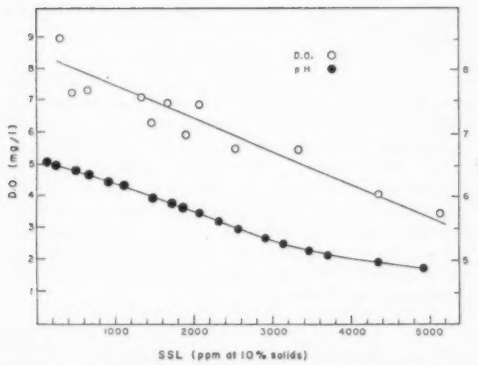

FIG. 1. The relationship between SSL levels, *p*H, and dissolved oxygen in aerated Exploits River water.

at an SSL concentration of 5150 ppm. If it had been desired to test only for the chemical toxicity of SSL, such a low oxygen level would have been unsatisfactory, although it was probably not low enough to directly cause death of the test fish (Doudoroff and Shumway 1970). Nevertheless, oxygen depletion in high SSL concentrations is inevitable, and it is felt that a bioassay procedure that attempted to ignore this aspect of the toxicity would be quite lacking in realism. Alderdice and Brett (1957) considered this point in stating that the interaction of effluent toxicity and lowered oxygen availability is a better criterion than toxicity alone for assessing the responses of young salmon to bleached kraft mill effluents. The *p*H minimum probably was of less importance, since Atlantic salmon have been found to occur naturally in waters with a *p*H as low as 5 (DeCola 1970).

Fish under stress in SSL solutions showed a very definite series of symptoms, much the same as has been previously noted in a study on four species of Pacific salmon (Williams et al. 1953). The first noticeable effect was a darkening of the dorsal integument, partially or completely obliterating the parr marks. This occurred at a concentration as low as 475 ppm, but not in lower concentrations or control tanks. The next noticeable effect, at concentrations over 1000 ppm, was loss of reaction to the sudden movement caused by lifting screen lids on the bioassay tanks. The next symptom to occur, as concentrations increased, was rapid and irregular breathing. At concentrations above 2500 ppm, the edges of the fins became white and very brittle. Death was preceded by loss of orientation, a period of immobility and very rapid breathing on the bottom of the tank, and, finally, a series of spasms in which the ventral and pectoral fins appeared to be paralyzed.

Treatment of the data from individual test tanks follows the procedure suggested by Sprague (1969). The toxicity curves and the time taken for 50% of the test fish to die (TD50's), are shown in Fig. 2. Figure 3 shows the response of the 96-hr LC50 to SSL concentration. The first experiment did not include concentrations high enough to kill 50% of the fish in any tank; accordingly, the results are shown only as points indicating surviving fish.

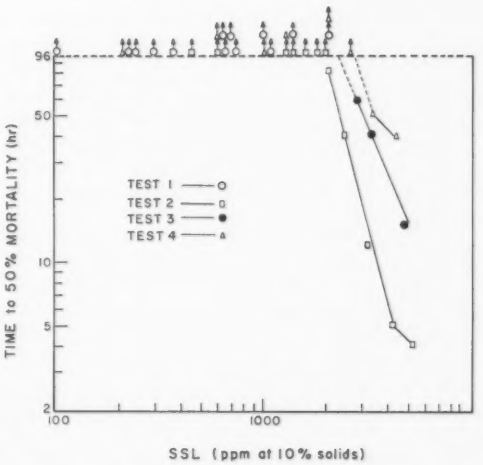

FIG. 2. The toxicity curve for SSL in aerated Exploits River water. The dashed lines indicate extrapolations to the end of the tests.

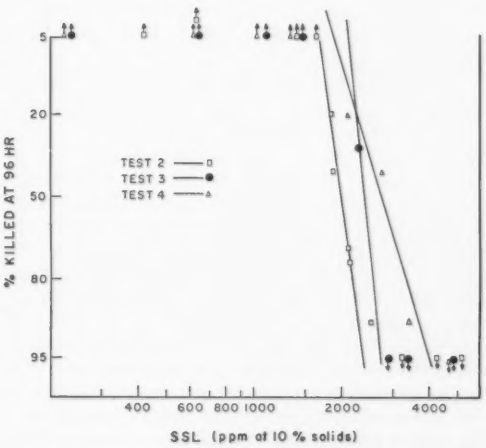

FIG. 3. The estimation of the LC50 for fresh and oxidized SSL in aerated Exploits River water.

Statistical features of the lines estimating the LC50's, according to the method of Litchfield and Wilcoxon (1949), are:

	Test		
	2	3	4
LC50 (*ppm*)	2000	2400	2750
Upper limit (*ppm*)	2096	2551	3080
Lower limit (*ppm*)	1908	2258	2455
Slope function	1.11	1.08	1.25

Within the range of experimental results, the lines shown in Fig. 3 for tests 2 and 3 are parallel at the 95% level, while the line for test 4 is not. However, the LC50 for each line is significantly different. These inferences imply that, while the response of test fish to the two different batches of fresh SSL was the same, the LC50 of each batch was different. However, for tests 3 and 4, both the response and the LC50 were different; oxidation of SSL, therefore, increased the LC50, presumably by altering the levels of one or more of the toxic components, but changed the way in which the test fish reacted to it. The 5-day oxidation of SSL under near-optimum conditions, which would seldom be achieved on a large scale, only decreased its toxicity by 14.5%.

The variation of toxicity between different batches of SSL is not surprising. Even within the same mill, the composition of such a heterogenous mixture as SSL might be expected to vary significantly with such factors as the type of wood being processed, the initial composition of the cooking liquor, and the temperature and duration of the pulp cook. Between mills, a much larger variation might logically be expected, especially in view of the large variation found in the toxicity of different kraft mill effluents by Howard and Walden (1971).

The 96-hr bioassay appears to be becoming a standard tool in the measurement of pulp mill effluent toxicities. However, 30-day continuous flow bioassays on Pacific salmon (Williams et al. 1953; Holland et al 1960) have shown that the incipient LC50, the level at which 50% of the test fish would survive after an indefinite exposure, is much lower than the 96-hr LC50. The results reported here for Atlantic salmon fall within the range of 96-hr LC50's reported for the four species of Pacific salmon, and it appeared that some fish exposed to a concentration less than the 96-hr LC50 were adversely affected by SSL at the termination of the tests. Some of these fish did not recover after being placed in clean water. Until further data are available, it is suggested that the application of results from 96-hr bioassays on sulphite mill effluents to environmental situations be made with more than the usual caution.

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Effects of Eyestalk Removal and Ecdysterone Infusion on Molting in *Homarus americanus*[1]

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FLINT, R. W. 1972. Effects of eyestalk removal and ecdysterone infusion on molting in *Homarus americanus*. J. Fish. Res. Bd. Canada 29: 1229–1233.

Techniques of inducing precocious molting by introduction of the steroid ecdysterone were studied on the American lobster, *Homarus americanus*. The results indicate that ecdysterone, at the dosages used, while accelerating premolt processes, sufficiently disrupts the normal sequence of molting. Destalked intermolt stage C lobsters were infused with ecdysterone, in quantities ranging from 1–6 μg/g body weight. Proecdysis was shortened, particularly in lobsters receiving more than 2 μg/g body weight of the steroid. They reached ecdysis 13.8 days (average) after infusion but showed no increase in limb bud growth and died during or immediately after ecdysis. Lobsters with eyestalks infused with Ringer's solution (3% NaCl), ethyl alcohol, β-Sitosterol, or ecdysterone failed to shorten their intermolt duration near 13.8 days.

It appears that, in *H. americanus*, removal of both eyestalks does not induce precocious molt as in other decapod crustaceans, but this animal exhibits a limb bud growth during the intermolt cycle, similar to what other investigators have found for other decapods.

FLINT, R. W. 1972. Effects of eyestalk removal and ecdysterone infusion on molting in *Homarus americanus*. J. Fish. Res. Bd. Canada 29: 1229–1233.

L'auteur examine les divers aspects de la mue précoce provoquée chez le homard américain, *Homarus americanus*, par l'injection du stéroïde ecdystérone. Les résultats suggèrent que les dosages d'ecdystérone employés, tout en accélérant le processus de prémue, perturbent l'ordre normal de la mue. Des doses d'ecdystérone variant de 1 à 6 μg/g de poids corporel ont été injectées dans des homards au stade C d'intermue, après ablation des pédoncules oculaires. Ceci a eu pour effet de raccourcir la proecdysie, surtout chez les homards qui avaient reçu plus de 2 μg de stéroïde par g de poids corporel. Les homards ont atteint le stade d'ecdysie 13.8 jours (moyenne) après injection, mais ne montrèrent aucun signe de croissance des bourgeons appendiculaires et moururent durant ou immédiatement après la mue. Les homards à pédoncules oculaires intacts, dans lesquels on avait injecté une solution de Ringer (3% NaCl), de l'alcool éthylique, du β-sitostérol ou de l'ecdystérone, n'ont pas raccourci leur période d'intermue.

Il semble donc que chez le homard, contrairement aux autres crustacés décapodes, l'ablation des deux pédoncules oculaires ne provoque pas la mue précoce. Par contre, cette espèce, comme les autres décapodes, exhibe un croissance des bourgeons appendiculaires durant l'intermue, phénomène déjà observé par d'autres chercheurs.

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UNLIKE many of the decapod crustaceans, lobsters do not appear to molt precociously after surgical removal of their eyestalks (Aiken 1969; Travis 1951). This may indicate that the molt-inhibiting hormone proposed to be found in the eyestalks in most decapod crustaceans (Passano 1951; Bliss and Welsh 1952) may have another site in lobsters, in addition to or exclusive of the eyestalks. Since the location of this site seems very difficult to determine, another approach was used to induce precocious molt in the American lobster, namely, the infusion of hormones.

Several investigators (Lowe et al. 1968; Krishnakumaran and Schneiderman 1970) have made use of the insect molting hormone ecdysone and its analogues to induce ecdysis, or precocious signs of ecdysis, in many Arthropods. The investigation of the effect of addition of the steroid ecdysterone (β-ecdysone) to lobsters was therefore carried out in

[1]The results reported here are a portion of a thesis submitted in partial fullfillment of the requirements for the Master of Science degree at Long Island University, Greenvale, N.Y.

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an attempt to upset the balance of molt stimulating–molt inhibiting hormones. Since eyestalk removal alone in *Homarus* was not found to result in shortened intermolt, the ecdysterone infusions were carried out on both unoperated and destalked animals.

Materials and Methods

Male and female lobsters (carapace length, 54–88 mm) from Long Island Sound were held separately in tanks supplied with 10 gal of aerated, filtered, and recirculated sea water at about 24 ppt salinity, an average temperature of 17 C, and a 12 hr light and 12 hr dark photoperiod. All animals were fed weekly with liver chunks.

To determine if the lobster limb bud growth is similar to that of *Gecarcinus lateralis* (Bliss 1956), the lobsters were induced to autonomize their third right walking legs and limb bud growths were measured through an entire intermolt cycle. The phase of the intermolt cycle for each animal before the study began was determined by Drach's (1939) classification. Only animals estimated to be in early C stage intermolt were used. Following limb bud removal, each lobster was further checked as to intermolt stage by progress of its limb bud growth (Bliss 1956).

The following treatment was used to prepare for eyestalk removal: early intermolt animals were placed in a tank of sea water containing 3.6 ml/liter of isobutyl alcohol, which served as an anesthetic (Foley et al. 1966). Each lobster was then placed on an operating board and one eyestalk removed, the second eyestalk being removed 24 hr later by the same procedure as the first. After each surgery, the lobster was replaced in filtered sea water for recovery. Spacing the removal of eyestalks prevented high mortality (Edwards 1950), which was about 20% in the present experiments. Gelfoam No. 12 (Upjohn Co.) was used to prevent excessive bleeding after surgery.

After removal of eyestalks and a recovery period of 24 hr, the animals were grouped as follows: (1) no further treatment; (2) ecdysterone infusions in varying amounts were given (1–6 μg/g body weight). This was done in a spring group of lobsters and again in a fall group. After infusion, all lobsters were replaced in their respective tanks and limb bud measurements were continued to ecdysis.

The technique of infusion was performed as follows. Three 20 ml syringes, containing the desired solutions were mounted on a Harvard Compact Infusion Pump. A Butterfly Infusion Set (Abbott Laboratories) with a 23-gauge needle was attached to each syringe. The needle was inserted into the pericardial sinus via a hole made in the dorsal posterior region of the carapace by a separate 23-gauge needle. Gelfoam No. 12 was packed around the inserted needle to support it and prevent bleeding. Each animal was placed in a tray containing enough aerated sea water to cover the gill area. The infusion pump was set to deliver 0.0333 ml/min over a 10-hr period.

The following controls were performed on early intermolt lobsters with eyestalks: (1) "infused" with needle only for several days; (2) infused with 20 ml of Ringer's solution (steril 3% NaCl: Stewart and Li 1969); (3) infused with 1–3 μg/g body weight of β-Sitosterol, a steriod found to be inactive in insect molting (Hsiao and Hsiao 1969); (4) infused with ethyl alcohol, the solvent of β-Sitosterol, and Ringer's solution; (5) infused with 1–4 μg/g body weight of ecdysterone in 20 ml of Ringer's solution.

Results

Limb bud growth curves similar to those for *Gecarcinus lateralis* (Bliss 1956) were observed in *Homarus americanus*. Initiation of proecdysis corresponded quite well with temperature increase (Fig. 1).

The intermolt durations of the three untreated lobsters and those subjected to anesthetic with eyestalk ablation or anesthetic alone are shown in Table 1. Removal of both eyestalks did not induce precocious molting and only one of six lobsters molted after 122 days, whereas 9 out of 10 with eyestalks molted, and six of these in a shorter period of time. The lobsters treated with anesthetic were not treated and measured until late January, whereas the untreated lobsters were begun in December.

Infusion of ecdysterone into destalked lobsters, both before the molting season[1] and after the peak of the season (Table 2) resulted in an apparent reduced intermolt duration, especially at higher dos-

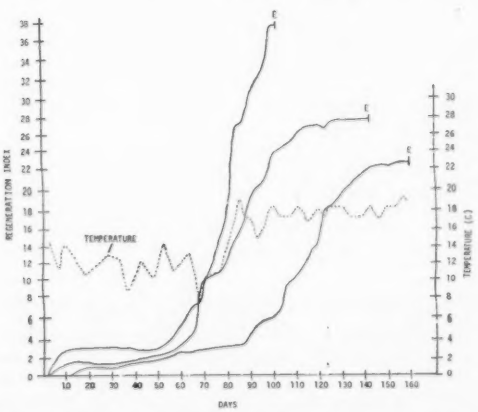

FIG. 1. Untreated lobster limb bud growth curves compared with average water temperature. E = ecdysis;

$$\text{Regeneration index} = \frac{\text{Length of limb bud (mm)}}{\text{Length of carapace (mm)}} \times 100$$

(Bliss 1956)

[1]Molting season determined from previous personal observations and from observations of local lobstermen.

ages. The larger concentrations of ecdysterone also appear to greatly retard limb bud regeneration. Figure 2 shows a representation of lobsters from both spring and fall experiments. The destalked lobsters receiving 3–6 μg/g body weight of ecdysterone (Fig. 2A) show no major proecdysial growth prior to ecdysis whereas those receiving 1–2 μg/g body weight (Fig. 2B) display a comparatively normal growth curve leading to ecdysis. Infusion of more than 2 μg/g body weight of ecdysterone into destalked lobsters, while decreasing intermolt, seems to interrupt certain metabolic pathways, retarding limb bud regeneration before ecdysis.

The β-Sitosterol infusions (Fig. 2C) were performed to determine if any steroid would give the results obtained from ecdysterone. In the fall group of experiments there was an obvious difference in limb bud growth between the lobsters with eyestalks receiving β-Sitosterol and those (with eyestalks) receiving ecdysterone. Figure 2D shows that ecdysterone-infused lobsters displayed a definite increase in proecdysial growth after infusion. Lobsters receiving β-Sitosterol did show an increase in limb growth, but it was minor when compared to ecdysterone and dissimilar compared to normal curves (Fig. 1). Although none of the fall lobsters with eyestalks in Table 2 reached ecdysis, the ecdysterone-infused animals did show precocious signs, such as increase in limb bud growth, reddening of the abdomen, and change in color of underlying epithelial pigments from brilliant orange-red to dark reddish-blue (indicative of apolysis), while the lobsters receiving β-Sitosterol displayed none of these signs. Also, the spring lobsters with eyestalks in Table 2 that received ecdysterone had a shorter intermolt than those receiving β-Sitosterol.

None of the destalked lobsters receiving ecdysterone lived more than a day after ecdysis. The fall lobsters (destalked) in Table 2 receiving 3–6 μg/g body weight of ecdysterone died at the onset of ecdysis without shedding their old exoskeletons.

TABLE 1. A comparison of limb bud growth and intermolt duration in unoperated and destalked winter *Homarus americanus*.[a]

Treatment	No. Lobsters	Duration of intermolt (days) (mean ± SE)	Lobsters reaching ecdysis
None	3	134.7±22.9	3
Anesthetic	7	101.0±14.1	6
	Bilateral eyestalk removal		
Anesthetic	6	122[b]	1

[a]Induced autotomy of third right walking leg in all lobsters.

[b]Only one lobster reached ecdysis; therefore, there is no average or standard error.

Discussion

The results of this study demonstrate that ecdysterone significantly shortens the intermolt time of destalked *H. americanus* and induces premature molt in spring and fall animals. This was most

TABLE 2. A comparison of limb bud growth and intermolt duration (mean ± SE) in unoperated and destalked spring and fall *Homarus americanus* infused with various control substances or with the hormone ecdysterone.[a]

Treatment	No. Lobsters	Final regeneration index	Duration of intermolt (days)	Lobsters reaching ecdysis
Infusion (10 hr): β-Sitosterol,	spring, 7	15.69±5.82	67.5±9.5	7
Ringer's sol'n, or ethyl alcohol	fall, 7	5.79±3.21	61.0[b]	0
Infusion (10 hr): ecdysterone	spring, 3	13.25±7.55	47.2±8.6	2
(1–4 μg/g body wt)	fall, 3	12.71±6.27	61.0[b]	0
Anesthetic, bilateral eyestalk removal, infusion (10 hr): ecdysterone	spring, 3	16.23±8.14	42.3±14.8	3
(1–2 μg/g body wt)	fall, 3	13.40±1.0	37.0±3.6	3
Anesthetic, bilateral eyestalk removal, infusion (10 hr): ecdysterone	spring, 3	2.15±0.27	14.7±1.0	3
(3–6 μg/g body wt)	fall, 3	2.64±0.54	13.0±2.7	3[c]

[a]Induced autotomy of third right walking leg in all lobsters.

[b]Fall control lobsters were not allowed to reach ecdysis before work was stopped.

[c]None were able to escape from their shell successfully before death, but all did approach ecdysis and separate their shell at the line of dishiscence.

striking in the case of the fall lobsters: they had molted in the laboratory approximately 1 month prior to treatment and molted again after the ecdysterone infusion. Ecdysterone alone, without eyestalk removal, was not as effective, but also shortened intermolt duration (Table 2).

The fact that the spring lobsters were further along in intermolt compared to the fall lobsters may explain why the fall destalked lobsters receiving 3–6 μg/g body weight of ecdysterone died during exuviation. Since they had recently molted, the higher dosages of ecdysterone, which caused another molt 13 days (average) after infusion, was apparently too great for the fall animals. The new exoskeleton might not have had enough time to form properly, causing it to remain attached to the old exoskeleton.

There is a great deal of confusion and speculation among invertebrate physiologists concerning the targets, site of synthesis, and mechanisms of the crustacean molting hormone, whether it be ecdysone, ecdysterone, or some other compound. The limb bud may be a target tissue of ecdysterone because the lobsters (with eyestalks) that received ecdysterone also displayed an increase in limb bud growth while not actually molting (Fig. 2D). This did not occur to as great an extent with the β-Sitosterol; however, the spring animals infused with β-Sitosterol did show shortened intermolt durations compared to those animals in Table 1. The exact role of eyestalks, however, can not be evaluated at this time. It also cannot be absolutely determined what effect ecdysterone or any other compound has

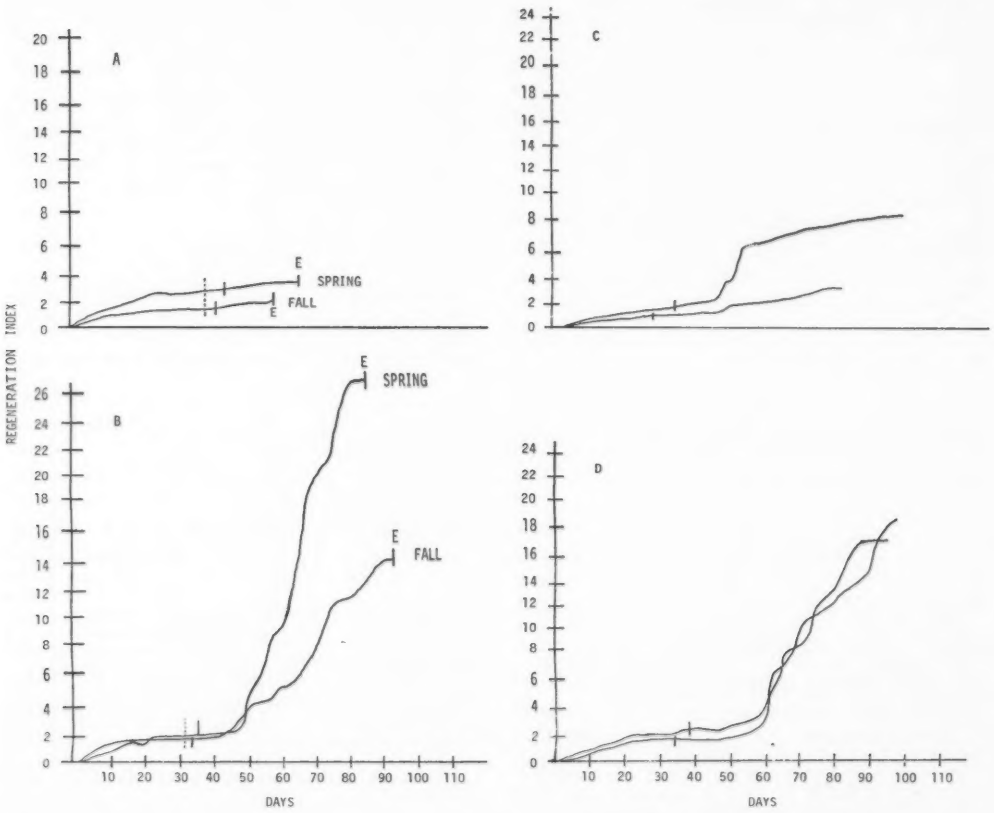

FIG. 2. A comparison between representative limb bud growths of destalked lobsters after infusion of (A) 3–6 μg/g body weight and (B) 1–2 μg/g body weight of ecdysterone and of lobsters with eyestalks after infusion of (C) β-Sitosterol and (D) 1–4 μg/g body weight of ecdysterone. Broken vertical line represents time of eyestalk removal; solid vertical line, time of infusion; and E, time of ecdysis.

on *H. americanus*. In the case of the lobsters that received high concentrations of ecdysterone, coupled with eyestalk ablation, this appeared to reduce limb bud growth. This may have been from toxic side effects and may reflect the problems that occur when tampering with the fine balance of molt inhibiting–molt inducing hormones in nature.

The exact mechanism of hormonal effects on molting *H. americanus* needs to be investigated to determine whether or not ecdysterone is the true molting hormone of this species or simply is working as a mimic to effect the intermolt.

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Benthos Trapped Leaving the Bottom in Bideford River, Prince Edward Island

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THOMAS, M. L. H., AND E. JELLEY. 1972. Benthos trapped leaving the bottom in Bideford River, Prince Edward Island. J. Fish. Res. Bd. Canada 29: 1234–1237.

Inverted funnel traps, designed to catch animals swimming up from the bottom, set in Bideford River estuary, P.E.I., during the summer of 1969 caught numerous benthic and hyperbenthic invertebrates. Many crustaceans generally considered to be planktonic appeared to enter the substratum during the day and to leave it at night. Several polychaetes, some of which were not known to swim, were regularly collected. The average catch per square meter per day was 23 individuals, amounting to 0.08 g (decalcified dry weight).

THOMAS, M. L. H., AND E. JELLEY. 1972. Benthos trapped leaving the bottom in Bideford River, Prince Edward Island. J. Fish. Res. Bd. Canada 29: 1234–1237.

Des attrapes en forme d'entonnoir renversé, concues pour la capture d'animaux remontant du fond, placées dans l'estuaire de la rivière Bideford, Ile-du-Prince-Edouard, durant l'été 1969, ont capturé de nombreux invertébrés benthiques et hyperbenthiques. Plusieurs crustacés, généralement considérés comme planctoniques, semblent pénétrer dans le substrat le jour et le quitter la nuit. Plusieurs polychètes, dont quelques-uns n'étaient pas considérés comme nageurs jusqu'à présent, ont été capturés régulièrement. Les prises moyennes par m^2 par jour ont été de 23 individus pesant en tout 0.08 g (poids sec décalcifié).

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BIDEFORD River estuary in northwest Prince Edward Island has been the site of an intensive 5-year study of its benthos (Hughes and Thomas 1971; Thomas MS 1970). In an associated study, stomach contents of all common estuarine fish were analyzed and benthos found to form significant amounts of food, even in pelagic fish such as the smelt *Osmerus mordax* and the mackerel *Scomber scombrus* (Thomas MS 1970). Many benthos, particularly annelids and crustaceans, are known to ascend into the water column to feed or reproduce and should only become available to pelagic fish at such times. This experiment was designed to investigate the magnitude of this phenomenon and its diurnal pattern in an estuarine ecosystem.

Method — Benthos leaving the bottom were collected in inverted funnel traps (Fig. 1). The funnels were of transparent vinyl sheet supported on a frame of ¼-inch (6-mm) iron rod. The apex of each funnel was constructed from a transparent plastic funnel cut to leave an opening 1 cm in diameter. The opening discharged into a 500 ml-glass jar, whose plastic top was cut to fit around the funnel and glued to it. The jar also communicated with the interior of the funnel through two openings screened with 0.5-mm mesh. This allowed some water to leave the collecting jar when it was hauled, while air entered through the funnel apex, thus reducing specimen loss. Each trap covered 0.44 m^2 and was about 75 cm high,

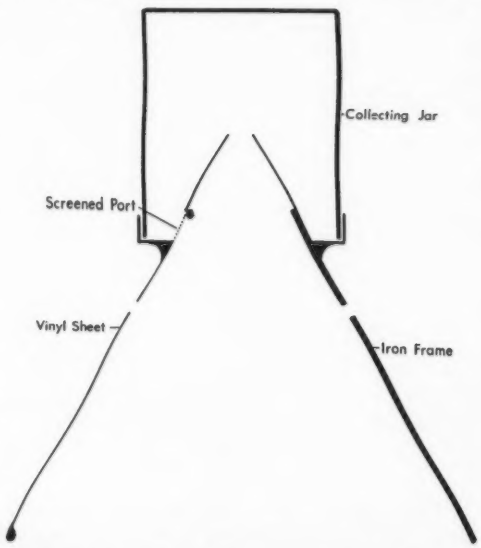

FIG. 1. Vertical section through funnel trap.

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enclosing 0.16 m³ of water. The design permitted no flow-through of water on setting that might mechanically trap fauna.

Five traps were usually set simultaneously in water less than 1.5 m deep and bedded in by hand; in deeper water they were lowered slowly on ropes. Divers confirmed that a good seal with the bottom was normal. Catches were discarded if turbid water was present in the collecting jar, indicating possible disturbance, or if a trap had moved while set. Traps were mostly set from 0900 to 1600 hr and from 1600 to 0900 hr the following day; some were set for 24-hr periods. Eighteen sets were out only during daylight and 19 were out predominantly at night.

When raised, traps were allowed to drain briefly and then inverted to remove the bottle, which was usually still almost full of water.

Catches were sorted alive and only swimming species considered. Each species was counted and weighed with surface water removed. Occasional samples were decalcified where appropriate and reweighed after drying in a forced air oven at 100 C to estimate the approximate dry organic matter.

Results — Traps were set at eight stations between July 25 and September 25, 1969. Table 1 lists general bottom and benthic characteristics for each station, the total number of hours traps were set at each one, and the mean catch per square meter per hour. Table 2 lists the 19 species of swimming invertebrates caught.

Traps were set on 49 occasions for varying periods, covering a total of 811 hr. Table 3 lists numbers and live weights per square meter per day for all invertebrate species taken in five (10%) or more sets. The calculated dry weight catch was 0.08g/m² per day. For *Carinogammarus mucronatus* and *Corophium insidiosum*, which were only present locally, calculations were made only on a basis of station of occurrence.

The mysid *Neomysis americana* contributed the greatest numbers, while the decapod *Crangon septemspinosus* supplied the greatest weight.

Great differences in daytime and nighttime catches were observed for many common species. Results expressed in catches per hr are presented in table 4. Standard deviations of numbers caught in each period are given where appropriate, together with significant data (*t*-tests were performed on log-transformed data — McIntyre 1971). The following species were taken only at night: *Cerebratulus lacteus*, *Ampelisca vadorum*, *Glycera dibranchiata*, *Carinogammarus mucronatus*, *Nereis succinea*, *Corophium insidiosum*, *Pherusa affinis*, *Crangon septemspinosus*, *Scoloplos fragilis*, *Palaeomonetes vulgaris*, *Gammarus* sp.

The invertebrates caught were much more active by night. Fourteen of 21 species were taken only in night time sets, or in significantly greater numbers in these.

Although the traps were not designed to catch plankton, and could not do so quantitatively, there were at times dense concentrations in the collecting jars. These were mostly copepods, but occasionally large numbers of hydroid medusae were present.

Several limitations must be placed on conclusions drawn from trap catches. Traps were not entirely efficient in that capture required an ascent of about 75 cm, which may be above the limit for some weakly swimming species. Some trapped fauna inevitably escaped from the collection jars during raising and others were consumed by predators also trapped. It is also impossible to conclude that the trapped specimens were necessarily within or on the bottom when the traps were installed.

Discussion — More invertebrates were collected over soft, smooth bottoms than elsewhere. These areas predominate but have comparatively low diversity of benthic species and a low benthic biomass composed mainly of infauna (Thomas MS 1970; Hughes and Thomas 1971).

TABLE 1. General benthic characteristics of stations, trap time at each, number of species caught, and mean numbers of invertebrates caught (number of sets in parentheses).

Station	General benthic characteristics	Total hr traps set	No species caught	No. invertebrates caught per m²/hr
1	Mud with eelgrass	90(2)	2	0.16
2	Mud with scattered eelgrass	179(9)	11	0.42
3	Mud with *Macoma baltica*	65(5)	9	0.58
4	Shelly-mud, mussel bed	96(4)	11	1.39
5	Mud with *Yoldia limatula*	103(9)	5	1.35
6	Sandy mud with eelgrass	48(3)	6	0.24
7	Muddy shell, oyster bed	103(8)	15	0.31
8	Mud with *Yoldia limatula*	127(9)	7	2.82

The biomass, abundance, and diversity of trapped fauna greatly exceeded expectations. Particularly surprising was the consistent presence of many crustaceans normally considered to be hyperbenthic. The design of the traps does not preclude the possibility that part of the catch was in the water rather than the sediment when they were set. If this was so, however, their failure to avoid capture is surprising. Despite the fact that many of these species are abundant in the area, they have neither been commonly caught in epibenthic sleds nor observed by divers. This evidence indicates that many of these species bury themselves by day and emerge at night. Herman (1963) thought that the mysid *Neomysis americana*, which was common in trap catches, lived on or just above the bottom by day. Kos (1969) found several *Calanus* species, which are typically planktonic, in the benthos. The frequency of occurrence of such behavior could only be ascertained using specially designed collecting apparatus.

Other species collected, principally of polychaetes, are typically benthic. The two *Nereis* species, *virens* and *succinea*, are well known for their pelagic spawning behavior (Pettibone 1963; Berkeley and Berkeley 1953). However, swimming is evidently not confined to spawning individuals, as immature specimens were common in the Bideford River collections (sexually mature *N. virens* were collected as late as early September, somewhat extending their reported breeding season [Pettibone 1963]). Pettibone (1963) also reported swimming activity in *Eteone lactea*, but did not associate it with spawning. Many of the specimens of this species taken in Bideford River were mature and spawned on handling. The other polychaetes collected, with the possible exception of *Glycera dibranchiata*, are not generally considered to be errant species. *Scoloplos fragilis*, which was taken regularly in the traps, is considered by Pettibone to be intermediate between errant and sedentary types and swimming activity has not been observed. *Pherusa affinis* is sedentary; only a single specimen was trapped. It is not abundant in the area.

The catch from the funnel traps averaged 0.08 g/m^2 per day (dry weight). This amount does not seem large, but, if extrapolated to the entire rather small Bideford River estuary, represents over 1000 kg/day (live weight) of benthic organisms available to pelagic feeders. Much of this biomass may return to the bottom, but the fact that Thomas (MS 1970) found many of these species to be important in the

TABLE 2. Species caught in inverted funnel traps in Bideford River, P.E.I., in 1969.

Species Caught
Nemertea
Cerebratulus lacteus (Leidy)
Annelida: Polychaeta
Eteone lactea Claparede
Glycera dibranchiate Leidy
Nereis succinea (Frey and Leukart)
Nereis virens Sars
Pherusa affinis (Leidy)
Scoloplos fragilis (Verrill)
Arthropoda: Crustacea
Ampelisca vadorum Mills
Ampithoe rubricata (Montagu)
Argulus sp.
Carinogammarus mucronatus (Say)
Corophium insidiosum Crawford
Crangon septemspinosus Say
Gammarus sp.
Mysis stenolepis Smith
Neomysis americana (Smith)
Neopanope texana (Smith)
Oxyurostylis smithii Calman
Palaemonetes vulgaris (Say)

TABLE 3. Percent frequency of capture, mean numbers, and live weights per square meter per day for common species collected in funnel traps.

Species or group	% frequency of capture	No.	Live weight (*g*)
All	80	23.04	0.341
E. lactea	31	0.86	0.004
N. succinea	10	0.23	0.013
S. fragilis	12	0.20	0.001
A. vadorum	10	0.09	0.001
A. rubricata	25	2.87	0.011
C. mucronatus	60	1.76	0.010
C. insidiosum	45	2.55	0.013
C. septemspinosus	41	1.05	0.222
N. americana	39	13.94	0.031
O. smithii	35	1.31	0.004
P. vulgaris	14	0.29	0.021

TABLE 4. Individuals of common species per square meter per hour caught in sets of funnel traps by night and day. Standard deviations are in parantheses.

Species	Day catches no.	Night catches no.
All	0.056(0.087)	0.790(0.760)[a]
Eteone lactea	0.021(0.048)	0.041(0.043)
Amphithoe rubricata	0.010(0.025)	0.053(0.074)[b]
Neomysis americana	0.008(0.023)	0.437(0.797)[a]
Oxyurostylis smithii	0.014(0.037)	0.168(0.285)[c]

[a]Significant at $P = <0.01$.
[b]Significant at $P = <0.05$.
[c]Significant at $P = <0.02$.

diet of pelagic estuarine fishes indicates that they form a significant food source.

Acknowledgments — I am most grateful to Dr R. L. Wigley of the U. S. Fish and Wildlife Service, Biological Laboratory, Woods Hole, Mass., who kindly checked the identifications of cumaceans and mysids. A. Michael and B. Morris, both of the Institute of Oceanography, Dalhousie University, Halifax, N.S., identified *Ampithoe rubricata* and some of the planktonic copepods, respectively; I thank them. Most of the plankton collected was identified by the Canadian Oceanographic Identification Centre, National Museum of Natural Sciences. Thanks are due to Dr D. J. Faber, Head, and Dr Chang-Tai Shih, Scientist-in-charge, zooplankton laboratory, for their help.

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Downstream Migration of Recently Transformed Sea Lampreys Before and After Treatment of a Lake Michigan Tributary with a Lampricide[1]

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HODGES, J. W. 1972. Downstream migration of recently transformed sea lampreys before and after treatment of a Lake Michigan tributary with a lampricide. J. Fish. Res. Bd. Canada 29: 1237–1240.

After the Pere Marquette River was treated with a lampricide in May 1964, the number of recently transformed sea lampreys (*Petromyzon marinus*) collected in the water-intake structure of a chemical plant near the mouth of the stream dropped 99.5%, from 13,913 (average for 1962–63 and 1963–64) to 76 (average for the next four migration seasons). Average length of the lampreys caught increased markedly after the treatment. In five of the six migration seasons, the catch of downstream migrants was higher in the fall than in the spring.

HODGES, J. W. 1972. Downstream migration of recently transformed sea lampreys before and after treatment of a Lake Michigan tributary with a lampricide. J. Fish. Res. Bd. Canada 29: 1237–1240.

Après traitement de la rivière Père Marquette avec un lampricide, en mai 1964, le nombre de grandes lamproies marines (*Petromyzon marinus*) nouvellement métamorphosées, capturées dans la prise d'eau d'une usine chimique près de l'embouchure, est passé de 13,913 (moyenne des saisons 1962–63 et 1963–64) à 76 (moyenne des quatre saisons de migration suivantes), soit une diminution de 99.5%. La longueur moyenne des lamproies capturées a augmenté notablement après le traitement. Au cours de cinq des six saisons de migration, les captures de lamproies descendant le cours d'eau ont été plus élevées en automne qu'au printemps.

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[1]Contribution 451, Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, Ann Arbor, Mich. 48107. This study, carried out while the author was a member of the Laboratory staff, was part of the sea lamprey program conducted by the Service under contract with the Great Lakes Fishery Commission.

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RECENTLY transformed sea lampreys, *Petromyzon marinus*, from the Pere Marquette River, Mich., were collected from January 1962 through December 1968 before and after treatment of the stream with the selective lampricide, 3-nitrofluoro-4-nitrophenol (TFM). Information was sought on differences in number and size of the migrants attributable to the treatment and on dates and duration of the annual downstream migrations.

The collections were from the catch basket of the water-intake structure of the Dow Chemical Co. plant at Pere Marquette Lake. The lake is near the mouth of the Pere Marquette River, a tributary of east-central Lake Michigan. Although the water intake is about ½ mile below the point at which the river enters Pere Marquette Lake, the use of fluorescein dyes has shown that the stream retains its identity as its flows along the shore of the lake, past the intake. Water enters the structure at a continuous rate of about 43 cfs. It passes through a 6-ft-wide grating of upright steel bars spaced 2 inches apart and is then pumped through two revolving drums covered with ¼-inch mesh screen. Debris from the screens is washed into a catch basket, where lampreys and other aquatic organisms are held alive and intact. The basket was emptied weekly during most of the year and as often as daily during certain peak periods in the migration season (primarily October–April). Water discharge was recorded at a U.S. Geological Survey gauging station, 12 miles upstream.

All lampreys were identified, and large samples of newly transformed sea lampreys were measured. The total catch included 28,267 recently transformed, 109 large adult, and 4 larval sea lampreys; 19 American brook lampreys (*Lampetra lamottei*); 240 chestnut lampreys (*Ichthyomyzon castaneus*); 4 northern brook lampreys (*I. fossor*); and 62 larval *Ichthyomyzon* (not identifiable to species).

The Pere Marquette River was treated with TFM on May 20–28, 1964, and May 12–17, 1968, by the standard procedures described by Applegate et al. (1961).

Number and size of newly transformed sea lampreys — The average annual catch of downstream migrants (Table 1) dropped 99.5% after treatment of the stream in May 1964, from 13,913 (average for 1962–63 and 1963–64) to 76 (average for the four subsequent migration seasons, 1964–65 through 1967–68).

The annual average length of recently metamorphosed sea lampreys caught in the catch basket increased from 134–142 mm before the TFM treatment to 153–164 mm in the migratory seasons following treatment (Table 1). A gradual increase in length of newly transformed downstream migrants was also reported for the Carp Lake River during a period (1948–49 to 1954–55) when spawning runs were blocked (Applegate 1961). Presumably the reduced density of the residual populations led to the increased growth in both streams.

TABLE 1. Numbers and average total lengths of recently transformed sea lampreys taken from the catch basket of a water-intake structure in Pere Marquette Lake, 1962–68[a].

Period[b]	No. caught	No. measured	Avg. length (*mm*)
1962	129	97	142
1962–63	7939	2115	136
1963–64	19,887	7728	134
1964–65	80	46	153
1965–66	69	67	156
1966–67	37	37	164
1967–68	120	119	162
1968	6	5	163

[a]Pere Marquette River was treated with TFM May 20–28, 1964, and May 12–17, 1968.

[b]The first period was January 1–April 30, 1962, and the last was May 1–December 31, 1968; all others extended from May 1 to April 30.

Migration season — In both 1962–63 and 1963–64 the migration season (Table 2) began in late October (only an occasional newly transformed sea lamprey was taken in the intake structure in May–August, and none in September); catches rose sharply to a yearly peak in late November, fell to low levels in January and February, and increased to a second peak (pronounced in 1962–63 but small in 1963–64) in March. By late April, catches had subsided to almost nil. Of the total catch during the two seasons, 71% in 1962–63 and 91% in 1963–64 were taken in the fall (October–December), and about 39% in 1962–63 and 72% in 1963–64 were taken November 16–30. Increases in the catch of migrants commonly accompanied or immediately followed increases or fluctuations in water level. A similar relation between lamprey movement and water level was reported for the Carp Lake River (Applegate 1961).

Although collections in the catch basket of the intake structure were small after the chemical treatment of the stream, the seasonal movements indicated by the 1962–63 and 1963–64 collections remained distinguishable. The total numbers of newly transformed ammocetes caught in the different years and (in parentheses) the percentages of these numbers taken in October-December were as follows: 1964–65, 80 (40); 1965–66, 69 (96); 1966–67, 37 (94); 1967–68, 120 (78). Thus the number of newly transformed sea lampreys taken in the catch basket was higher in the fall (October–December) than in the "spring" (January–April) in 5 of 6 years.

The apparent predominance of movement in the fall was apparently more common in the Pere Marquette River than in the Carp Lake River,

TABLE 2. Numbers of newly transformed sea lampreys taken from the catch basket during different periods, and discharge of the Pere Marquette River in 1962–63 and 1963–64.

Dates	No. collections	River discharge (*cfs*)		Sea lampreys caught	
		Avg.	Range	No.	% of season's total[a]
		1962–63			
May 1–Sept. 30	14	538	398–970	7	0.1
Oct. 1–15	1	453	–	0	–
Oct. 16–31	1	528	–	61	0.8
Nov. 1–15	3	501	480–518	185	2.3
Nov. 16–30	7	487	462–502	3085	38.9
Dec. 1–15	4	485	470–509	822	10.4
Dec. 16–31	2	560	540–580	1482	18.7
Jan. 1–15	6	542	450–580	183	2.3
Jan. 16–31	3	507	500–520	34	0.4
Feb. 1–15	3	470	450–480	25	0.3
Feb. 16–28	3	483	470–500	27	0.3
Mar. 1–15	4	532	480–570	40	0.5
Mar. 16–31	12	1191	596–2270	1966	24.8
Apr. 1–15	5	1201	679–1760	19	0.2
Apr. 16–30	2	652	638–665	3	tr
Total	70	684	398–2270	7939	–
		1963–64			
May 1–Sept. 30	21	488	346–1180	7	tr
Oct. 1–15	2	373	366–380	1	tr
Oct. 16–31	3	400	376–416	28	0.1
Nov. 1–15	11	447	434–470	1254	6.3
Nov. 16–30	15	539	451–688	14,395	72.4
Dec. 1–15	15	504	470–558	2381	12.0
Dec. 16–31	5	454	450–460	45	0.2
Jan. 1–15	4	482	470–490	98	0.5
Jan. 16–31	9	570	480–660	349	1.8
Feb. 1–15	7	493	450–550	184	0.9
Feb. 16–29	4	476	460–504	30	0.2
Mar. 1–15	14	772	524–930	811	4.1
Mar. 16–31	14	637	556–782	125	0.6
Apr. 1–15	11	686	545–831	177	0.9
Apr. 16–30	1	764	–	2	tr
Total	136	554	346–930	19,887	–

[a]Tr = <0.05.

where downstream migrations were observed for 10 seasons, from 1948–49 to 1957–58. Applegate and Brynildson (1952) reported that most movement was in the spring in 1948–49, 1949–50, and 1950–51, and data presented by Applegate (1961) showed that a higher percentage of the season's total catch was taken in the spring than in the fall in 1954–55 and 1955–56; in the other five migration seasons the percentage was slightly to markedly higher in the fall.

Acknowledgments — The employees in the powerhouse and plant protection departments of the Dow Chemical Company, Ludington, Mich., first called attention to the lampreys at the intake and allowed personnel of the U.S. Fish and Wildlife Service to make the collections. The entire staff of the Service's Ludington Biological Station participated in the collection of lampreys and data. D. Pettengill, U.S. Geological Survey, Grayling, Mich., provided the stream discharge data. V. C. Applegate and the late N. S. Baldwin read the manuscript and made suggestions.

APPLEGATE, V. C. 1961. Downstream movement of lampreys and fishes in the Carp Lake River, Michigan. U.S. Fish Wildl. Serv. Spec. Sci. Rep. Fish. 387: 71 p.

APPLEGATE, V. C., and C. L. BRYNILDSON. 1952. Downstream movement of recently transformed sea lampreys, *Petromyzon marinus*, in the Carp Lake River, Michigan. Trans. Amer. Fish. Soc. 81: 275–290.

APPLEGATE, V. C., J. H. HOWELL, J. W. MOFFETT, B. G. H. JOHNSON, AND M. A. SMITH. 1961. Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 1: 35 p.

CORRECTIONS TO RECENT PUBLICATIONS
OF THE
FISHERIES RESEARCH BOARD OF CANADA

Journal of the Fisheries Research Board of Canada

VOLUME 28, No. 5 — paper by K. Ostovar, R. R. Pereira, and R. A. Gallop: *Effects of gamma irradiation on Salmonella spp. in smoked lake whitefish (Coregonus clupeaformis).*

Page 643: line 23, "merlan" should be "coregone."

VOLUME 28, No. 11 — paper by D. W. Schindler, F. A. J. Armstrong, S. K. Holmgren, and G. J. Brunskill: *Eutrophication of Lake 227, Experimental Lakes Area, northwestern Ontario, by addition of phosphate and nitrate.*

Page 1772 Fig. 22, caption, "Dissolved silicate μg Si/liter, 1969," should read "Dissolved silicate, mg Si/liter, 1969."

VOLUME 28, No. 12 — paper by J. R. Markert and W. E. Vanstone: *Egg proteins of coho salmon (Oncorhynchus kisutch): chromatographic separation and molecular weights of the major proteins in the high density fraction and their presence in salmon plasma.*

Page 1853: line 31, "Received April 20, 1970," should read "Received April 20, 1971."

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia

(4) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(5) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(6) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(7) Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(8) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

1675. STASKO, A. B. 1971. Review of field studies on fish orientation. Ann. N.Y. Acad. Sci. 188: 12–19. (6)

1676. WATSON, J. 1972. Ecdysis of the snow crab, *Chionoecetes opilio*. Can. J. Zool. 49: 1025–1027. (6)

Additional FRB Publications

The Fisheries Research Board of Canada also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at Board establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

L'Office des recherches sur les pêcheries du Canada publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements de l'Office situés a Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal Fisheries Research Board Canada

Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

Editorial Practices

Abstracts should be provided in both English and French.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. NW., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopédique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year. The *Journal* follows the spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Journal of the Fisheries Research Board of Canada

Guide des auteurs

Des détails sur le tapage et certains autres item sont donnés dans le "Guide pour les secrétaires d'auteurs." Aussi, une liste d'épellations recommandées d'environ 100 mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication.

Disposition générale

Les résumés doivent être fournis en anglais et en français.

Le *Style Manual for Biological Journals* (deuxième édition, publié par l'American Institute of Biological Sciences, 2000, rue *P* N.-O., Washington, D.C.), a été adopté comme guide des auteurs. Le *Third New International Dictionary* de Webster, dans le cas de l'anglais, et le *Grand Larousse Encyclopédique*, dans le cas du français, font respectivement autorité pour l'orthographe et la séparation des mots. Une exception est faite à l'égard des travaux en anglais qui se conforment entièrement à l'*Oxford English Dictionary*. Les titres de périodiques sont abrégés selon la *Biological Abstracts*. Toute référence à la littérature doit suivre le système nom-année. Les rapports manuscrits sont inclus dans la liste des références, les lettres "MS" avant l'année les identifient dans les références et dans le texte. Le *Journal* se sert des épellations recommandées dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No 6 de l'American Fisheries Society.

Les *notes* se distinguent des écrits d'après l'étendue de la recherche plutôt que par la qualité de la recherche. Les notes peuvent être sur des travaux de recherche de portée restreinte, susceptibles de découler d'investigations plus considérables, pour la description de techniques de recherche et de méthodes de pêche, pour l'établissement de priorités de publication, et pour faire connaître les renseignements utiles tirés d'entreprises qui ont été interrompues avant d'avoir été achevées. Si la portée de la Note le permet, elle peut être disposée comme un mémoire. D'autre part, la disposition est flexible. La longueur ne doit pas dépasser quatre pages imprimées.

En général, les mémoires en série doivent être évités.

Les illustrations

Les dessins au trait doivent être exécutés à l'encre de chine sur papier à dessin blanc ou sur papier à calquer, ou sur papier à coordonnées avec quadrillage bleu. Les lignes, les symboles, les lettres et les chiffres doivent tous être propres. Ils doivent être lisibles quand ils sont réduits à la grandeur voulue pour la reproduction désirée. Les dessins doivent être groupés, si la chose est possible, de façon à utiliser tout l'espace disponible. Les originaux doivent être soumis et devraient mesurer moins de 20 pouces dans le sens le plus long pour les illustrations de pages entières, et proportionnellement plus petit pour les illustrations de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves des photographies doivent être tirées sur papier glacé et accuser une gamme étendue de teintes. Elles doivent être coupées, dressées et montées, au moyen de dissolution, sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Epreuves et tires a part

Les épreuves en placards sont adressées à l'auteur et la responsabilité de toute erreur qui peut y rester lui incombe.

Cinquante tirés à part, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander d'autres tirés à part.

Liste de contrôles pour manuscrits

Une attention particulière portée sur les questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs. Les mêmes questions sont recommandées aux arbitres qui font la critique des livres pour le *Journal*.

Generalites

Est-ce que les connaissances nouvelles du rapport méritent d'être publiées?

Les découvertes, les interprétations et les conclusions sont-elles solides et pertinentes au but de l'étude?

Si le manuscrit est tout simplement corroboratif, mérite-t-il d'être publié?

Le manuscrit doit-il être récrit avant d'évaluer le travail?

La contribution est-elle placée dans une perspective appropriée relative à l'état des connaissances du sujet?

Le manuscrit est-il trop long?

Les tables et figures sont-elles toutes nécessaires et arrangées pour économiser le plus d'espace?

Le texte serait-il plus clair s'il était condensé?

Est-ce qu'une partie des données devrait être disponible séparément, soit dans un rapport ou dans un dépôt des données?

Le manuscrit devrait-il être condensé en une note?

Est-ce que la préparation du manuscrit suit la logique mentionnée dans l'introduction?

Y a-t-il des exposés sommaires au début de chaque section et paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents aux sujets?

Y a-t-il des contradictions entre les tableaux et les figures du texte, ou dans le texte même?

Si le manuscrit est tiré d'une thèse, contient-il des caractéristiques communes à cette dernière, mais indésirables dans un journal?

PARTIES DU MANUSCRIT

Le titre renferme-t-il le contenu du rapport?

Le résumé donne-t-il l'essentiel des connaissances nouvelles?

L'introduction a-t-elle tous les points importants afin d'orienter le lecteur?

Le renvoi bibliographique se limite-t-il à la définition du problème?

Les détails du matériel et des méthodes employées sont-ils limités à un minimum pour que les scientifiques comprennent le but de l'étude et puissent porter un jugement adéquat sur les données obtenues.

Les généralisations sur les résultats sont-elles adéquates?

Les conclusions sont-elles déduites des résultats?

La discussion est-elle limitée à l'interprétation et la signification de ces découvertes?

S'il y a des échappatoires dans l'interprétation, sont-elles reconnues? Devraient-elles être maîtrisées?

Devrait-on accepter d'autres interprétations?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables

Examine the current volume of the *Journal* to see how tables are set up (Appendix C).

Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

Illustrations

Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas

Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References

Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings

A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices

Appendices A to F are available on request from the Office of the Editor.

Journal
Fisheries
Research
Board
Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des auteurs" dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de taper ou d'arranger les détails, suivez la pratique du *Journal*.

Tapez le manuscrit sur un papier blanc et non un papier pelure, mesurant de préférence 8½ × 11 po. (21.5 × 28 cm) et laissant une marge d'au moins 1 po. (2.5 cm). Si possible, employez un papier avec des lignes numérotées dans la marge. Employez un ruban nylon ou carbone et gardez les clés du dactylo nettes.

Chaque partie du manuscrit doit être tapée à double-interligne au moins. La chose est particulièrement importante dans le cas des tableaux, légendes d'illustrations, renvois au bas de la page et listes de références bibliographiques. Evitez de souligner à moins que la matière ne soit en italique. Mettez en retrait la première ligne de chaque paragraphe du texte, ainsi que de chaque légende et renvoi.

Dans les titres et en-têtes, n'employez la majuscule que pour le premier mot, les noms propres et les adjectifs. Pour les petites majuscules, utilisez les petites lettres.

Indiquez tous les renvois au texte par des chiffres arabes en-tête. Numérotez-les consécutivement à travers le texte en commençant par ceux de la page-titre. Placez chaque renvoi immédiatement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit donner que le titre du sujet, le nom et l'affiliation de l'auteur ou des auteurs, et tout autre renvoi utile (Appendice A). Tapez le résumé sur une page séparée (Appendice B). Numérotez chaque page, débutant avec la page-titre.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et un double du travail au Directeur de la publication.

Tableaux

Consultez une copie du *Journal* pour savoir composer les tableaux et placer les lignes qui y entrent (Appendice C).

Portez une attention particulière à ce qui suit concernant les *tableaux numérotés*.

Tapez les tableaux sur des pages séparées, en utilisant du papier sans lignes numérotées.

Numérotez les tableaux avec des chiffres arabes.

Employez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, et très rarement ailleurs. N'employez jamais de lignes verticales; de préférence, laissez de l'espace supplémentaire.

Indiquez les renvois dans les tableaux au moyen de petites lettres italiques en position supérieure et tapez-les au bas de la page.

Placez les tableaux après la liste de références.

A l'endroit où un tableau est mentionné pour la première fois, tapez dans la marge "Tableau (- - -) près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, ils sont tapés avec le texte. Ils ont rarement besoin de lignes horizontales, et l'information est préparée de façons à prendre le moins de rangées possibles.

Illustrations

Joignez au manuscrit expédié le dessin original des illustrations et des photocopies en deux exemplaires.

Au bas et à gauche de chaque figure et photocopie, inscrivez le nom de l'auteur et le numéro de la figure. Aussi, tapez chaque légende avec sa figure pour fin d'identification. Pour les photographies, tapez l'information sur une lisière de papier et collez-la au dos de la photographie. N'écrivez pas et ne tapez pas sur la photographie même. Tapez ensemble sur une feuille séparée et par ordre consécutif les légendes des illustrations, et intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, tapez dans la marge "Fig. (- - -) près d'ici."

Equations, formules et expressions mathematiques

Laissez trois espaces au-dessus et au-dessous des lignes qui contiennent du matériel en positions supérieures ou en positions inférieures, et au-dessus et au-dessous d'équations et de formules. Dans les différentes expressions, placez chaque composante dans sa position appropriée (Appendice D).

Indiquez les symboles clairement. Dans la marge, épelez les lettres grecques la première fois qu'elles apparaissent et faites une distinction entre la lettre "l" et le chiffre "un" et entre la majuscule "O" et le

chiffre "zéro." Procédez de la même façon pour d'autres notes explicatives.

Références

Vérifiez le texte afin d'assurer que sont mentionnées toutes les références ou renvois de la liste. Tapez, à doubles interlignes et de la façon prévue (Appendice E) pour le *Journal*, la liste de références. Les noms de journaux sont abrégés suivant la *Biological Abstracts*, mais s'il y a doute, écrivez le nom au long.

Vocabulaire

Une liste d'environ cent mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication (Appendice F).

Appendices

Les appendices de A à F sont disponibles sur demande au bureau du Directeur de la publication.

Announcement

The Department of Indian Affairs and Northern Development requires an

Environmental Advisor

(salary up to $23,413)

for a 3-year term appointment to carry out the following duties in the Environmental/Social Program, Northern Pipelines:

- to be fully familiar with the various proposed pipeline routes
- to make detailed examinations, both in headquarters and the field of research projects (both government and industry) with emphasis on those dealing with hydrological, limnological, and fisheries aspects, and to assess the scientific value of each project
- to develop standard basic criteria for the evaluation of environmental projects (both government and industry) as to their respective contribution to the decision-making process with regard to the pipeline program
- to identify environmental problems and recommend on intensity of existing research and initiation of new projects
- to prepare, edit, evaluate, and comment on reports on environmental aspects of northern pipeline construction and operation
- to keep fully up to date with pertinent scientific and technical literature
- to participate in the preparation of environmental portions of submissions to the Cabinet and Treasury Board
- to assist in the preparation of the environmental portion of the final Program report
- to provide advice on the terms and conditions for the protection of the environment to be imposed on firms constructing and operating northern pipelines

Qualifications: Applicants should hold a Ph.D. degree in a field related to aquatic ecology or a lesser degree and demonstrated capacity to do independent research equivalent to that for a Ph.D. degree; plus extensive relevant work experience which preferably should include experience in inter-disciplinary environmental studies; evidence of ability and knowledge in the planning, management, and direction of scientific programs; ability in fostering and carrying out cooperative arrangements with and between other agencies; a thorough understanding of the problems involved in the field of work; personal suitability and satisfactory physical condition; knowledge of the English language is essential.

Apply to: *Public Service Commission of Canada*
Ottawa, Ontario K1A OM7
Att'n: *Program Director, Bio-Physical Sciences*
Staffing Branch

To ensure full consideration applications should be received by September 15, 1972 (quote file 72-1154).

Bulletin 167 *(Revised Edition)*

A Practical Handbook of Seawater Analysis

The original publication on this subject appeared under the title of A Manual of Sea Water Analysis in 1960 as Bulletin No. 125, Fisheries Research Board of Canada. A second edition was published in 1965 and both editions have been long out of print. The first edition of a completely revised and updated version was published under the title of A Practical Handbook of Seawater Analysis in 1968 as Bulletin No. 167, and went out of print in 1971.

This is the second edition of the latter work. It contains revised methods for determination of Ammonia Plus Amino Acids and of Ammonia, and one new method not in the first edition, "Determination of Urea." Minor corrections and alterations have been made throughout the text. These changes will add to the value of this text as an authoritative reference book on seawater analysis.

The senior author, Dr J. D. H. Strickland passed away in California on November 12, 1970. The second author, Dr T. R. Parsons, introduced the changes in this edition.

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